

# **EXHIBIT B**

**PATENT****IN THE UNITED STATES PATENT AND TRADEMARK OFFICE***In re* Application of:Issam RAAD, Hend A. HANNA, and Nabeel  
NABULSI

Group Art Unit: 1744

Serial No.: 10/044,842

Examiner: Jastrzab, Krisanne Marie

Filed: January 11, 2002

Atty. Dkt. No.: UTSC:669US

For: NOVEL ANTISEPTIC DERIVATIVES  
WITH BROAD SPECTRUM  
ANTIMICROBIAL ACTIVITY FOR THE  
IMPREGNATION OF SURFACES**SECOND DECLARATION OF DR. ISSAM RAAD UNDER 37 C.F.R. § 1.132**

I, Dr. Issam Raad, hereby declare as follows:

1. I am one of the inventors of the above-referenced patent application. I am a citizen of the U.S., currently residing at 4207 Clearwater Ct., Missouri City, TX, 77459.
2. The Example section of the above-referenced patent application provides strong evidence of synergy of gentian violet (GV) and chlorhexidine (CHX) as an antiseptic/disinfectant. I use the term "synergy" to refer a combined action of basic reagent and dye that is greater than the expected action of the basic reagent and dye separately. Table 2 and Table 3 on page 20 of the referenced patent application show zones of inhibition (ZOI) produced by coated endotracheal PVC tubes (using DCM or MeOH). As set forth in the application on page 20, lines 16-19, "endotracheal PVC tubes impregnated with Gendine (GN) are far more effective against all

organisms when compared with those impregnated with CHX, and more effective than PVC tubes impregnated with GV against *Pseudomonas aeruginosa*."

3. Table 4 on page 21 of the referenced application shows ZOI produced by coated silicone catheters. Page 21, lines 10-12 states that "data in Table 4 shows how silicone catheters impregnated with GN are more effective in inhibiting *MRSA*, *PS* and *C. parapsilosis* than catheters impregnated with either GV or CHX."

4. Table 5, on page 21 of the present application, shows ZOI produced by coated polyurethane catheters (PU). Page 21, lines 24-27 states that "PU catheters impregnated with GN are more effective than PU catheter impregnated with GV in inhibiting *Pseudomonas aeruginosa*, and more effective than PU catheters impregnated with CHX against all three organisms, *MRSA*, *PS* and *C. parapsilosis*."

5. Table 6, on page 22 of the present application, shows ZOI produced by coated silk sutures. Page 21, lines 10-12 provides that "silk sutures coated or impregnated with GN are significantly more effective in inhibiting *MRSA*, *PS* and *C. parapsilosis* than sutures coated with either GV or CHX."

6. Similarly, Tables 7-10 on pages 24-25 show similar synergy against various bacterial and fungal organisms, when GV was combined with other basic reagents on the surfaces of medical devices.

7. Furthermore, as discussed in my first declaration (filed with the response to the Office Action dated January 11, 2006), I have provided additional evidence demonstrating that the combination of a basic reagent and a dye has antiseptic ability as a mouthwash, coating of a glove, or coating of a catheter than is more than additive compared to either dye alone or basic reagent alone (see Exhibit 1 of my first declaration).

8. In addition, attached as Exhibit 1 of this declaration is a summary of data from my laboratory that further demonstrates a high level of synergy of the combination of a basic reagent and a dye in antiseptic ability.

9. The most serious forms of catheter related bloodstream infections are those caused by fungi, particularly *Candida albicans*. This is the infection with the highest mortality rate – around 40%. We have found that gendine (GV and CHX) mixed in a specific molar ratio to coat catheters and devices provides unexpectedly superior synergy against *Candida albicans*. The strain used in the studies summarized in Exhibit 1 was obtained from a patient who suffered from catheter-related fungemia/candidemia caused by *Candida albicans* (strain 009-3072). In the first part of the study summarized in Exhibit 1, we calculated a minimal inhibitor concentration (MIC) and minimal fungicidal concentration (MBC) for each of the components, GV and CHX. The MIC/MBC was 0.5 microgram per mL for the GV and 16 microgram per mL for CHX.

10. When we tested for synergy of the combination of CHX and GV over a range of 1:1 to 100:1, and we obtained the results described on page 2 of Exhibit 1. Boxes that are shaded had a complete kill of the *Candida albicans* at the respective concentrations of the components that are

lower than the MIC and MBC of CHX alone and GV alone. The best synergistic data was obtained at a ratio of CHX:GV of 1:1 and 10:1, with a plateauing effect at 25:1 and thereafter. In other words, there is synergy obtained at 50:1 and 100:1 but it is not appreciably different from 25:1.

11. These results clearly establish that the claimed methods using a combination of a dye and basic reagent are surprisingly and unexpectedly superior compared to methods of disinfecting using dye alone or basic reagent alone.

12. Further, my group has published a study (Bahna *et al.*, Oral Oncol. 2007 Feb.; 43(2):159-64; Exhibit 2) that demonstrates that mouthwash compositions that have a ratio of dye:basic reagent of 10:1-66:1 demonstrated synergistic antimicrobial efficacy against free-floating and biofilm forms of *Candida albicans*.

13. I hereby declare that all statements made by my own knowledge are true and all statements made on information and belief are believed to be true and further that statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment under § 100 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date 4/20/07

Issam Raad

Issam Raad

APR. 20. 2007 8:27AM

M. D. ANDERSON ID/IC

NO. 358 P. 6

# **EXHIBIT 1**

## Efficacy and Potency of Gendine and its components against *Candida albicans*.

### Objective:

1. To determine potency (MIC, MBC) for the components of Gendine (Gentian Violet and Chlorhexidine) as well as optimal concentration of both components mixed together to form Gendine.
2. To determine potency (MIC, MBC) for the molar ratios of Gendine (Chlorhexidine:Gentian Violet).

### Materials and Methods:

#### *Potency of Gendine, Gentian Violet and Chlorhexidine –*

Using standard techniques to determine MIC and MBC, gentian violet and chlorhexidine were tested independently against *Candida albicans* (009-3072).

#### *Potency of Molar Ratios of Gendine*

Using standard techniques to determine MIC and MBC, molar ratios (25:1, 10:1, and 1:1) of the combination of gentian violet and chlorhexidine were tested against *Candida albicans* (009-3072).

### Results:

#### *Potency of Gendine, Gentian Violet and Chlorhexidine –*

		CA 009-3072
Gentian Violet	MIC ( $\mu\text{g/mL}$ )	0.5
	MBC ( $\mu\text{g/mL}$ )	0.5
Chlorhexidine	MIC ( $\mu\text{g/mL}$ )	16
	MBC ( $\mu\text{g/mL}$ )	16



*Potency of Molar Ratios of Gendine –*

Red = Kill, Black = Growth

Possible synergistic effect of Gendine is shown highlighted in yellow. (kill at concentrations below both individual MIC)

All Ratios shown are CHX:GV

Experiment - November 3, 2006

**C.albicans 009-3072**

	1	2	3	4	5	6	7	8
+ control								
100:1	MHB	0.192:0.0016	0.384:0.0032	0.767:0.0064	1.534:0.013	3.069:0.026	6.14:0.051	
50:1	MHB	0.192:0.0032	0.384:0.0064	0.767:0.013	1.534:0.026	3.069:0.051	6.14:0.103	
25:1	MHB	0.192:0.0064	0.384:0.013	0.767:0.026	1.534:0.051	3.069:0.103	6.14:0.205	
10:1	MHB	0.157:0.0064	0.315:0.013	0.632:0.026	1.264:0.103	2.527:0.205	5.055:0.41	
1:1	MHB	0.063:0.051	0.126:0.103	0.253:0.205	0.505:0.41	1.01:0.82	2.02:1.64	4.04:3.28
								8.08:6.56

CHX:GV	MIC
100:1	6.14 : 0.051
50:1	6.14 : 0.103
25:1	6.14 : 0.205
10:1	2.527 : 0.205
1:1	0.253 : 0.205

CHX MIC 16 GV MIC 0.5

# **EXHIBIT 2**



## Antiseptic effect of a novel alcohol-free mouthwash: A convenient prophylactic alternative for high-risk patients

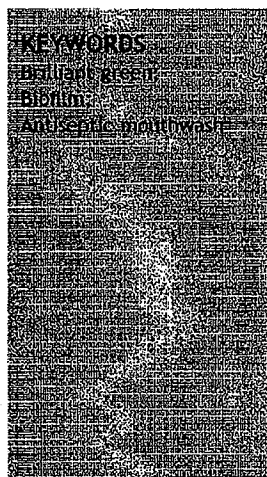
Paul Bahna<sup>a</sup>, Hend A. Hanna<sup>a,\*</sup>, Tanya Dvorak<sup>a</sup>, Ara Vaporciyan<sup>b</sup>,  
Mark Chambers<sup>c</sup>, Issam Raad<sup>a</sup>

<sup>a</sup> Department of Infectious Diseases, Infection Control and Employee Health, The University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 402, Houston, TX 77030, United States

<sup>b</sup> Department of Thoracic and Cardiovascular Surgery, The University of Texas, M.D. Anderson Cancer Center, TX, United States

<sup>c</sup> Department of Dental Oncology, The University of Texas, M.D. Anderson Cancer Center, TX, United States

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**Summary** We developed an efficacious and non-irritant mouthwash that is alcohol-free and that has a low concentration of chlorhexidine, in order to be used for preventing oral cavity infections in immunocompromised and cancer patients. The novel mouthwash solution was tested for its antimicrobial efficacy against both free floating (planktonic) and the biofilm forms of *Candida albicans*. The solution was also tested against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* (MRSA), using a modification of a previously published method. The activity of the novel mouthwash was also compared with that of three commercially available mouthwashes. The experimental mouthwash showed efficacy against *C. albicans*, both in free-floating form and in biofilm. With higher concentration of chlorhexidine, the solution was also efficacious in inhibiting the growth of *K. pneumoniae*, *P. aeruginosa*, and MRSA. The antiseptic activity of the alcohol-free mouthwash against other bacterial organisms and *C. albicans* was comparable to other commercially available alcohol-based mouthwash solutions. A novel alcohol-free mouthwash solution, that has low concentration of chlorhexidine, showed antiseptic effect against planktonic and biofilm forms of *C. albicans* and against *K. pneumoniae*, *P. aeruginosa*, and MRSA.

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\* Corresponding author. Tel.: +1 713 792 7943; fax: +1 713 792 8233.  
E-mail address: [hhanna@mdanderson.org](mailto:hhanna@mdanderson.org) (H.A. Hanna).

## Introduction

In critically ill and immunocompromised patients, the oral cavity is a common colonization site for numerous multidrug resistant bacterial and fungal microorganisms that can cause infections. Oral candidiasis is highly prevalent among immunocompromised patients and patients with dentures.<sup>1-3</sup>

There has been an increase in the number of immunocompromised patients, over the past few decades, caused in part by the rise in the numbers of bone marrow and solid organ transplantations, the increasing number of patients needing critical care, and the aggressive use of chemotherapy and radiation therapy.<sup>4</sup> Oral mucositis is recognized as a common complication of radiation therapy in patients with head and neck carcinoma, and currently, its treatment is essentially palliative.<sup>5</sup> Additionally, *Candida*-associated stomatitis is also a recognized complication in elderly denture users, especially when denture hygiene is lacking.<sup>6</sup> Epstein et al. found out that oropharyngeal colonization by *Candida* species was common in recipients of hematopoietic cell transplants, despite systemic and topical antifungal prophylaxis.<sup>7</sup> They also showed that patients who underwent total-body irradiation and who had evidence of *Candida* colonization were at higher risk of death after their transplant than those who were *Candida*-negative ( $P < .001$ ).<sup>7</sup>

Furthermore, fungal microorganisms such as *Candida* species are among the most common microorganisms causing nosocomial bloodstream infections in the United States. Although *Candida* species are part of the normal mouth flora in 25–50% of healthy individuals, often referred to as asymptomatic colonization,<sup>8</sup> this rate tends to be higher in patients with debilitating diseases such as HIV patients,<sup>9</sup> diabetic patients,<sup>10</sup> and patients with cancer,<sup>11</sup> where *Candida* are more prone to cause symptomatic disease rather than mere asymptomatic colonization. Within five years of seroconversion, up to 26% of HIV positive patients develop oral candidiasis, which is seen also in 12–100% of cancer patients undergoing chemotherapy, according to a published analysis of 15 studies, and in 76% of patients undergoing bone marrow transplant, and in up to 77% of carefully followed-up asthmatics using inhaled corticosteroids.<sup>12-15</sup>

Bacterial microorganisms can either migrate or be aspirated from the oropharynx throughout the respiratory system, and cause pneumonia. In addition, the microorganisms can form biofilm along the surface of an endotracheal tube and develop into infection involving the respiratory airway, leading to ventilator-associated pneumonia. An estimated 300,000 ventilator-associated pneumonia occur in the United States annually, with a mortality rate of at least 33%.<sup>16</sup> Some data advocate the pre-operative use of antiseptic mouthwash to prevent or minimize the occurrence of pulmonary infections.<sup>17</sup>

Most mouthwashes contain either a high concentration of chlorhexidine (0.12%) or some alcohol. Mouthwashes, containing chlorhexidine or alcohol, were shown to promote a significant reduction in microbial load in the oral cavity.<sup>18</sup> Chlorhexidine is one of the most effective antimicrobial agents for controlling dental plaque, but is known to have a bitter taste. On the other hand, alcohol is a potent

antimicrobial but is also irritating, especially to sensitive or inflamed mucosa. Both factors are of concern to the oral care of cancer patients, who are immunocompromised due to chemotherapy, and often suffer from mucositis, and hence would benefit from the use of a mild efficacious mouthwash. Unfortunately, there are few mouthwashes that are suitable for this group of patients, in terms of comfort and safety.

Chlorhexidine is a known antiseptic substance that is widely used in commercial mouthwash and other antiseptics. It is an efficacious antimicrobial agent for the control of dental plaque. Brilliant green is a triphenyl methane dye with an antiseptic activity when used in high concentration.

Brilliant green is used in high concentration, along with gentian violet and proflavine hemi sulfate, in the triple dye broad-spectrum antiseptic solution for neonatal nursery use.<sup>19-22</sup> The objective of the study was to develop an efficacious antimicrobial alcohol-free mouthwash that is non-staining, non-irritant, and that would be useful for the oral care of immunocompromised patients, such as patients with cancer, HIV patients, and those on steroids. We tested the solution against *Candida albicans*, a common cause of fungal infections in cancer patients, as well as against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and MRSA, all being common sources of infection in immunocompromised patients. We also tested the efficacy of three other commercially available mouthwashes against these organisms.

## Materials and methods

The model we used is based on the methods outlined in the federal registry for antiseptic drug products,<sup>23</sup> and which are commonly used to assess and compare antimicrobial activity of different oral antiseptic mouthwashes. The novel mouthwash tested, contained chlorhexidine and brilliant green, and is referred to in this manuscript as Gardine.

We tested the efficacy of Gardine against the free-floating form of *C. albicans*, *K. pneumoniae*, *P. aeruginosa*, and MRSA, and against the biofilm form of *C. albicans* (for adherence). We compared its efficacy with that of the following alcohol-based mouthwashes: PerioGard® (Colgate-Palmolive, Canton, MA), which contains 11.6% alcohol, Listerine® (Warner-Lambert, Morris Plains, NJ), which contains 26.9% alcohol, and Scope® (Procter & Gamble, Cincinnati, OH), which contains 14.3% alcohol.

## Preparation of the mouthwash solution

We used chlorhexidine in different low concentrations; 0.006% for testing the free-floating organisms, 0.012%, 0.024% for testing *C. albicans* biofilm, and 0.04% for the comparison with other mouthwashes. Chlorhexidine was combined with a low staining concentration of 0.004 mg/mL of brilliant green dye. The mouthwash solution was prepared by mixing chlorhexidine gluconate solution with brilliant green powder that was previously dissolved in distilled water. We also used brilliant green at two concentrations of 0.004 mg/mL and 0.008 mg/mL.

### Mouthwash activity against planktonic organism

After preparing the antiseptic solutions, inoculums of  $1.5 \times 10^8$  colony forming units/mL (CFU/mL), made of a 0.5 McFarland standard, was prepared for the *C. albicans*, *K. pneumoniae*, *P. aeruginosa* and MRSA. A 500  $\mu$ L of the prepared inoculum were then added to 500  $\mu$ L of Gardine antiseptic solution and incubated at 37 °C for 10 min. A 100  $\mu$ L were then pipetted and plated onto Trypticase soy agar (TSA) with 5% sheep blood plates and incubated at 37 °C overnight. Colonies were then counted up to 100 then multiplied by the dilution factor of 50 and documented.

### Mouthwash activity against *Candida* in biofilm

We used a modification of a previously published bioprosthetic biofilm colonization model.<sup>24</sup> As a surface for biofilm adherence, we used discs, 20 mm in diameter and 2 mm in thickness, made of the same material as denture (mixture of polyacrylic powder and liquid acrylic monomer) that were manufactured at the dental laboratory of M.D. Anderson Cancer Center. We used artificial saliva without enzymes (to avoid any antibacterial effect) as a medium (Roxane Laboratories, Inc. Columbus, OH). Discs were soaked in saliva and were incubated overnight at 37 °C. Dental discs were removed from the saliva and placed into 50 mL tubes (6–7 pieces/tube) and covered with inoculum (~10 mL), equal to McFarland standard, and were incubated overnight at 37 °C in a shaker incubator. Inoculated broth was removed with pipettes; 10 mL of saline was added and incubated for 30 min, as a washing step, to get rid of any free-floating organisms. Discs were then removed gently and placed into separate clean tubes containing two Gardine solutions, prepared with two different chlorhexidine concentrations: 0.012% and 0.024%, as well as saline, as control, and were left to soak for 10 min in the shaker incubator at 37 °C. The antiseptic solution was then removed and the dental discs were transferred into fresh tubes, each containing 5 mL of 0.9% saline. The tubes were sonicated for 5 min, then vortexed for 30 s, to detach any remaining colonies from the surface of the discs. Then 100  $\mu$ L were pipetted from each tube and spread on blood agar plates, and incubated overnight at 37 °C. For the control, the same steps were taken, using broth instead of the antiseptic solution. Colonies in each plate were counted up to 100,

multiplied by the dilution factor 50, and findings were documented.

### Comparison of Gardine's activity with other alcohol-containing mouthwashes

Gardine solution was prepared, using a higher concentration of chlorhexidine (0.04%) and 0.004 mg/mL of brilliant green. Equal amounts were obtained of three commercially available, alcohol-containing mouthwashes: Periogard®, Listerine®, and Scope®. Four inocula ( $1.5 \times 10^8$  CFU/mL) made of a 0.5 McFarland standard, were prepared of four microorganisms: *C. albicans*, *K. pneumoniae*, *P. aeruginosa*, and MRSA. A volume of 500  $\mu$ L of each of the prepared inoculum were then added to 500  $\mu$ L of each antiseptic solution and incubated at 37 °C for 10 min; 100  $\mu$ L were then pipetted and plated onto TSA blood plates and incubated at 37 °C overnight. The number of colonies in the plate was counted up to 100 then multiplied by the dilution factor 50 and results were documented.

## Results

### Activity against free-floating organisms

Gardine, at its low concentration was active against the free-floating *C. albicans*, *K. pneumoniae*, and MRSA. Brilliant green alone, at a concentration of 0.004 mg/mL and 0.008 mg/mL was not active against all organisms, also chlorhexidine alone, at two low concentrations of 0.006% and 0.012%, produced a partial or no antimicrobial activity. However, Gardine solution inhibited the growth of the organisms, indicating a possible synergistic effect of brilliant green with the low concentration of chlorhexidine (Table 1).

### Activity against *Candida* in biofilm

Gardine solution was active against the biofilm form of *C. albicans*. There was a complete eradication of *C. albicans* biofilm after being exposed to Gardine (0.008 mg/mL brilliant green and 0.012% chlorhexidine) and Gardine (0.008 mg/mL brilliant green and 0.024% chlorhexidine) solutions (Table 2).

Table 1. Synergism of chlorhexidine and brilliant green in solutions towards three free floating microorganisms

	<i>Klebsiella pneumoniae</i> CFU	MRSA CFU	<i>Candida albicans</i> CFU
Saline (control)	5000	5000	5000
0.004 mg/mL brilliant green	5000	5000	5000
0.004 mg/mL brilliant green	5000	5000	5000
0.006% chlorhexidine	5000	5000	100
0.004 mg/mL brilliant green + 0.006% chlorhexidine	0	0	0
0.008 mg/mL brilliant green + 0.006% chlorhexidine	0	0	0
0.012% chlorhexidine	0	5000	100
0.008 mg/mL brilliant green + 0.012% chlorhexidine	0	0	0

The test solution (0.008/0.012%) was tested against MRSA only, expressed as CFU/disk.  
 CFU = colony forming units, MRSA = methicillin resistant *Staphylococcus aureus*.

Table 2 Synergism of chlorhexidine and brilliant green in solutions against *Candida albicans* biofilm, expressed as CFU/disk

Solution	<i>Candida albicans</i>		
	CFU		
Saline (control)	5000	5000	5000
0.002 Brilliant green	5000	5000	5000
0.012 Chlorhexidine	5000	5000	5000
0.024 Chlorhexidine	5000	5000	5000
0.002 Brilliant green + 0.012 chlorhexidine	0	0	0
0.002 Brilliant green + 0.024 chlorhexidine	0	0	0

CFU = colony forming units; MRSA = methicillin-resistant *Staphylococcus aureus*

### Comparing Gardine activity with other alcohol-containing antiseptic mouthwashes

Gardine solution, prepared with 0.04% chlorhexidine, was active against free-floating forms of the four organisms tested, *C. albicans*, *P. aeruginosa*, *K. pneumoniae*, and MRSA. Gardine solution was as active as Periogard® and Listerine® mouth rinse solutions in completely eradicating the free-floating organisms, as shown on petri dishes by the absence of microorganisms' growth, while Scope® mouth rinse showed some growth with MRSA and *P. aeruginosa* (Table 3).

### Discussion

This in vitro study showed that the experimental mouthwash, while being free of alcohol and containing reduced concentration of chlorhexidine (0.04%), was shown to be efficacious in inhibiting bacterial and candidal activity. Gardine, was as active as two of the three tested commercial mouthwashes, and more active than the third, against the free-floating *C. albicans*, *P. aeruginosa*, *K. pneumoniae*, and MRSA, which are common causes of nosocomial pneumonia and ventilator-associated pneumonia, while being alcohol-free and possibly with a less bitter taste than some available products, due to its low content of chlorhexidine. The solution also proved effective against *Candida* embedded in biofilm.

Investigators in a recent study,<sup>25</sup> found pre-intubation gargling with povidine-iodine solution to be effective in reducing the post-intubation nosocomial pneumonia, caused by general bacteria and MRSA colonization in the pharynx. In patients with cancer and those who are immunocompromised, however, the povidine-iodine solution could be very irritating. In another study, chlorhexidine gluconate 0.12% oral rinse was found to be useful in reducing nosocomial pneumonia in patients undergoing heart surgery.<sup>26</sup> The overall rate of nosocomial pneumonia was reduced by 52% in the chlorhexidine 0.12%-treated patients compared to Listerine® treated patients, while among patients intubated for more than 24 h, pneumonia rate was reduced by 58% ( $P=0.06$ ). Unfortunately, such level of chlorhexidine concentration (0.12%) could be irritating to critically ill and immunocompromised patients, who suffer from oral cavity ulcers and mucositis, in addition to its unpleasant taste. Ventilator-associated pneumonia is addressed in a CDC report, whereby it is emphasized that patients receiving mechanical ventilation are at highest risk for acquiring nosocomial pneumonia.<sup>27</sup> The use of antiseptic mouthwash, as part of a multifaceted protocol implemented in the intensive care unit, contributed to reducing the incidence of ventilator-associated pneumonia by about 50% ( $P < 0.0001$ ).<sup>28</sup> In an in vivo study, oral colonizing bacteria increased significantly after rinsing with a sucrose solution. The oral bacteria were not affected by rinsing with water or fluoride mouth rinse, but were significantly inhibited by rinsing with chlorhexidine, cetylpyridinium chloride or triclosan/copolymer dentrifice. A dose-dependent inhibition was noted with chlorhexidine rinses.<sup>29</sup> Chlorhexidine can directly damage the microbial cytoplasmic membrane as well as it can attach to a variety of substrates, allowing for the antimicrobial property of the chemical.<sup>30</sup>

Breck et al. demonstrated, in a study of volunteers, that a combination of habitual self-performed and non-supervised oral hygiene with mouth rinse was found to be more beneficial for plaque control than the use of traditional oral hygiene alone. In addition, when mouth rinses were used to supplement routine oral hygiene, chlorhexidine was found to be the most powerful solution.<sup>31</sup>

However, while some authors report the potential benefit of chlorhexidine on oral mucositis and as an antimicrobial rinse, with antiplaque effects as well as antibacterial and antifungal activity, others report lack of impact against mucositis.<sup>32,33</sup> In a double blind randomized study of patients receiving radiation therapy of the oral cavity mucosa,

Table 3 Comparison between efficacy of alcohol-free Gardine solution and three alcohol-containing mouthwashes, against four microorganisms (free floating) expressed as CFU/disk

Solution	MRSA			<i>Pseudomonas aeruginosa</i>			<i>Klebsiella pneumoniae</i>			<i>Candida albicans</i>		
Saline (control)	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
0.004 Brilliant green	0	0	0	0	0	0	0	0	0	0	0	0
0.04% chlorhexidine	0	0	0	0	0	0	0	0	0	0	0	0
Periogard	0	0	0	0	0	0	0	0	0	0	0	0
Listerine	0	0	0	0	0	0	0	0	0	0	0	0
Scope	50	0	0	1000	0	0	0	0	0	0	0	0

CFU = colony forming units; MRSA = methicillin-resistant *Staphylococcus aureus*

chlorhexidine mouthwash was found to be associated with mouthwash-induced discomfort, taste alteration, and teeth staining, more than the placebo mouthwash.<sup>33</sup>

On the other hand, chlorhexidine was found to be beneficial in controlling some carcinogenic substances. A study concluded that chlorhexidine mouthwash significantly reduced salivary acetaldehyde production, a metabolite resulting from ethanol consumption and which has been shown to have multiple mutagenic effects and to be carcinogenic to animals.<sup>34</sup>

The three commercially available mouthwash solutions that were tested in this study contain alcohol in variable concentrations; PerioGard<sup>®</sup> has 11.6% alcohol, Listerine<sup>®</sup> has 26.9% alcohol, and Scope<sup>®</sup> has 14.3% alcohol. It should be noted that alcohol in mouthwash is contraindicated in certain high-risk patients, including those who have mucositis, those undergoing head and neck irradiation, those who are alcoholic, and those who are immunocompromised.<sup>35,36</sup> Winn et al. found an association between the risk of oral cancer and the frequent and prolonged use of alcohol-based mouthwashes with high alcohol contents. The risk of oral cancer increased by 40–60%, after adjusting for other risk factors, such as tobacco and alcohol consumption.<sup>37</sup> Hence it is preferable, if possible to avoid the use of mouthwash with high alcohol content. Eldridge et al. addressed earlier the antimicrobial efficacy of an alcohol-free chlorhexidine mouth rinse,<sup>38</sup> and found no difference between the commercial alcohol-based chlorhexidine 0.12% and the alcohol-free chlorhexidine 0.12% through both in vitro and in vivo studies. The Gardine solution could prove to be advantageous in that it is alcohol-free and contains lower chlorhexidine concentration.

Gram-negative bacteria, such as *K. pneumoniae* and *P. aeruginosa*, and Gram-positive bacteria, such as MRSA that frequently colonize the oral cavity of hospitalized patients,<sup>39,40</sup> have emerged as causes of nosocomial pneumonia. This has stimulated the search for preventive and therapeutic measures to minimize oral and respiratory colonization by the simple use of a broad-spectrum antiseptic mouthwash pre-operatively or pre-intubation.<sup>26,28</sup> In this study, Gardine solution proved effective against the free-floating forms of these bacteria.

In addition to mouth and respiratory tract colonization with multidrug-resistant bacteria, *Candida* infections have shown a substantial increase in the United States during the last two decades. This has contributed to the rise in prolonged hospitalization and related deaths. *Candida* species have been shown to be the fourth most common group of organisms causing nosocomial bloodstream infections in the United States.<sup>41</sup> Factors contributing to this trend include a growing population of immunocompromised patients, such as HIV, cancer, chronic use of steroids, and transplant patients, as well as the use of new aggressive and invasive therapeutic strategies such as irradiation.

Among immunocompromised patients and patients with cancer, candidiasis is more prevalent in patients with hematologic malignancies than in those with solid tumors and among neutropenic patients than otherwise.<sup>42</sup> The experimental antiseptic mouthwash, Gardine, provided coverage against *C. albicans*, and it may prove suitable for use in immunocompromised patients, providing a valuable protection against this opportunistic nosocomial infection.

This current study has several limitations, including those inherent to its in vitro design, such as the limitations of extrapolating from this in vitro study to intact mammalian systems, and hence the need for in vivo studies. While the study addressed the effectiveness of Gardine solution in preventing the free-floating forms of bacteria and fungi, as well as fungal biofilm formation, biofilm testing for bacteria needs to also be studied. Additional in vitro as well as in vivo studies should be performed to further modify the alcohol-free mouthwash solution so that it may provide a potent coverage to broader spectrum of the organisms that colonize the oral cavity. Although neither chlorhexidine alone nor brilliant green alone is cytotoxic, testing Gardine for cytotoxicity is important if it is to be considered for further use.

In conclusion, Gardine solution showed an antimicrobial activity against *C. albicans* and other common bacteria. Its efficacy against the biofilm-embedded *C. albicans* has also been shown. The antiseptic activity of Gardine demonstrated a comparable activity with other commercially available, alcohol-containing mouthwashes. The novel Gardine solution may serve as a convenient alternative mouthwash for immunocompromised cancer patients and for pre-operative patients at high risk for nosocomial pneumonia. Its low concentration of chlorhexidine may minimize the unpleasant taste, thus enhancing patient compliance. Furthermore, being alcohol-free makes it non-irritant, thus gentler to use for patients with sensitive or inflamed mucosa.

## References

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# **EXHIBIT C**

## ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

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(iii) Manuscripts describing new or novel methods or improvements in media and culture conditions will not be considered for publication in AAC unless these methods are applied to the study of problems related to the production or activity of antimicrobial agents. Such manuscripts are more appropriate for *Applied and Environmental Microbiology* or the *Journal of Clinical Microbiology*.

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Minireviews, Guest Commentaries, and Comment Letters to the Editor are not subject to page charges. New-Data Letters to the Editor are subject to page charges.

### Editorial Style

The editorial style of ASM journals conforms to the *ASM Style Manual for Journals* (American Society for Microbiology, 2007, in-house document) and *How To Write and Publish a Scientific Paper*, 6th ed. (Greenwood Press, Westport, CT, 2006), as interpreted and modified by the editors and the ASM Journals Department.

The editors and the Journals Department reserve the privilege of editing manuscripts to conform with the stylistic conventions set forth in the aforesaid publications and in these Instructions.

### Review Process

All manuscripts are considered to be confidential and are reviewed by the editors, members of the editorial board, or qualified ad hoc reviewers. To expedite the review process, authors **must** recommend at least three reviewers who are not members of their institution(s) and have never been associated with them or their laboratory(ies); please provide their contact information where indicated on the submission form.

*Copies of in-press and submitted manuscripts that are important for judgment of the present manuscript should be included as supplemental material to facilitate the review.*

When a manuscript is submitted to the journal, it is given a number (e.g., AAC00047-07 version 1) and assigned to one of the editors. (**Always refer to this number in communications with the editor and the Journals Department.**) It is the responsibility of the corresponding author to inform the coauthors of the manuscript's status throughout the submission, review, and publication processes. The reviewers operate under strict guidelines set

forth in "Guidelines for Reviewers" (<http://www.journals.asm.org/misc/reviewguide.shtml>) and are expected to complete their reviews expeditiously.

The corresponding author is notified, generally within 4 to 6 weeks after submission, of the editor's decision to accept, reject, or require modification. When modification is requested, the corresponding author must either submit the modified version within 2 months or withdraw the manuscript. A point-by-point response to the reviews must be provided in the designated section of the Rapid Review submission form for the revised manuscript, and a compare copy of the manuscript (without figures) should be included as supplemental material if the editor requested one.

Manuscripts that have been rejected, or withdrawn after being returned for modification, may be resubmitted if the major criticisms have been addressed. (**Note: A manuscript rejected by one ASM journal on scientific grounds or on the basis of its general suitability for publication is considered rejected by all other ASM journals.**) The cover letter must state that the manuscript is a resubmission, and the *former manuscript number should be provided* in the appropriate field on the submission form. A **point-by-point response** to the reviews and a compare copy of the revised manuscript showing the changes must be included as supplemental material (the Response to Reviews section appears in the submission form only if the manuscript is a modification). Resubmitted manuscripts are normally handled by the original editor.

**Rejected manuscripts may be resubmitted only once unless permission has been obtained from the original editor or from the editor in chief.**

### Notification of Acceptance

When an editor has decided that a manuscript is acceptable for publication on the basis of scientific merit, the author and the Journals Department are notified. A PDF version of the accepted manuscript is posted online as soon as possible (see below).

The text files undergo an automated preediting, cleanup, and tagging process specific to the particular article type, and the illustrations are examined. If all files have been prepared according to the criteria set forth in these Instructions and those in Rapid Review, the acceptance procedure will be completed successfully. If there are problems that would cause extensive corrections to be made at the copyediting stage or if the files are not acceptable for production, ASM Journals staff will contact the corresponding author. Once all the material intended for publication has been determined to be adequate, the manuscript is scheduled for the next available issue and an acceptance letter indicating the month of publication, approximate page proof dates, and table of contents section is mailed to the corresponding author; a copyright transfer agreement is also included, as is a license to permit posting of supplemental material (if applicable). The editorial staff of the ASM Journals Department completes the editing of the

manuscript to bring it into conformity with prescribed standards.

### Publish ahead of Print

For its primary-research journals, ASM posts online PDF versions of manuscripts that have been peer reviewed and accepted but not yet copyedited. This feature is called "[journal acronym] Accepts" (e.g., AAC Accepts) and is accessible from the Journals website. The manuscripts are published online as soon as possible after acceptance, on a weekly basis, before the copyedited, typeset articles are published. They are posted "as is" (i.e., as submitted by the authors at the modification stage) and do not reflect ASM editorial changes. **No corrections/changes to the PDF manuscripts are accepted.** Accordingly, there likely will be differences between the AAC Accepts manuscripts and the final, typeset articles. The manuscripts remain listed on the AAC Accepts page until the final, typeset articles are posted. At that point, the manuscripts are removed from the AAC Accepts page and become available only through links from the final, typeset articles. The manuscripts are under subscription access control until 4 months after the typeset articles are posted, when free access is provided to everyone (subject to the applicable ASM license terms and conditions). Supplemental material intended, and accepted, for publication is not posted until publication of the final, typeset article.

Instructions on how to cite such manuscripts may be found in the References section below (p. 11).

### Page Proofs

Page proofs, together with a query sheet and instructions for handling proofs, will be made available to the corresponding author electronically via a PDF file that can be accessed through a unique password. Since corresponding authors will be notified of the availability of their PDF proofs, instructed how to access information about page charges, reprints, and color figure charges (if applicable), and assigned their unique password via e-mail, an e-mail address **must** be supplied in the correspondent footnote. Failure to do so may result in a delay in publication. **The PDF page proofs must be printed out, and corrections must be written on the hard copy.** Queries must be answered on the query page or on a separate sheet of paper, and any changes related to the queries must be indicated on the proofs. Note that the copy editor does not query at every instance where a change has been made. Queries are written only to request necessary information or clarification of an unclear passage or to draw attention to edits that may have altered the sense. It is the author's responsibility to read the entire text, tables, and figure legends, not just items queried. As soon as the page proofs are corrected and signed by the person who proofread them (within 48 h), **they should be mailed or sent by a courier service** such as FedEx, **not** faxed or sent as an e-mail attachment, to the ASM Journals Department.

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## HOW TO SUBMIT MANUSCRIPTS

All submissions to AAC must be made electronically via the Rapid Review online submission and peer review system at the following URL: [www.rapidreview.com/ASM2/author.html](http://www.rapidreview.com/ASM2/author.html). (E-mailed submissions will not be accepted.) First-time users must create an Author account, which may be used for submitting to all ASM journals. Instructions for creating an Author account are available at the above URL under the Create Account button. Step-by-step instructions for submitting a manuscript via Rapid Review are available from the account holder's My Manuscripts page. Information on file types acceptable for electronic submission can be found under the More About File Formats button.

PDFs of submitted manuscripts are retained in Rapid Review for 1 to 2 years, after which they are deleted.

## ORGANIZATION AND FORMAT

On receipt at ASM, an accepted manuscript undergoes an automated preediting, cleanup, and tagging process specific to the particular article type. To optimize this process, manuscripts must be supplied in the correct format and with the appropriate sections and headings.

Type every portion of the manuscript double spaced (a minimum of 6 mm between lines), including figure legends, table footnotes, and References, and number all pages in sequence, including the abstract, figure legends, and tables. Place the last two items after the References section. **Manuscript pages should have line numbers; manuscripts without line numbers may be editorially rejected by the editor, with a suggestion of resubmission after line numbers are added.** The font size should be no smaller than 12 points. It is recommended that the following sets of characters be easily distinguishable in the manuscript: the numeral zero (0) and the letter "oh" (O); the numeral one (1), the letter "el" (l), and the letter "eye" (I); and a multiplication sign (×) and the letter "ex" (x). Do not create symbols as graphics or use special fonts that are external to your word processing program; use the "insert symbol" function. Set the page size to 8½ by 11 inches (ca. 21.6 by 28 cm). Italicize or underline any words that should appear in italics, and indicate paragraph lead-ins in bold type.

Authors who are unsure of proper English usage should have their manuscripts checked by someone proficient in the English language.

**Manuscripts may be editorially rejected, without review, on the basis of poor English or lack of conformity to the standards set forth in these Instructions.**

### Full-Length Papers

Full-length papers should include the elements described in this section.

**Title, running title, and byline.** Each manuscript should

present the results of an independent, cohesive study; thus, numbered series titles are not permitted. Exercise care in composing a title. Avoid the main title/subtitle arrangement, complete sentences, and unnecessary articles. On the title page, include the title, running title (not to exceed 54 characters and spaces), name of each author, address(es) of the institution(s) at which the work was performed, each author's affiliation, and a footnote indicating the present address of any author no longer at the institution where the work was performed. Place an asterisk after the name of the author to whom inquiries regarding the paper should be directed (see "Correspondent footnote" below).

**Study group in byline.** A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations if desired) of the contributing members may be given in a footnote keyed to the study group name in the byline or as a separate paragraph in Acknowledgments.

If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

**Correspondent footnote.** The complete mailing address, a single telephone number, a single fax number, and a single e-mail address for the corresponding author should be included on the title page of the manuscript. This information will be published in the article as a footnote to facilitate communication, and the e-mail address will be used to notify the corresponding author of the availability of proofs and, later, of the PDF file of the published article.

**Abstract.** Limit the abstract to **250 words or fewer** and concisely summarize the basic content of the paper without presenting extensive experimental details. Avoid abbreviations and references, and do not include diagrams. When it is essential to include a reference, use the same format as shown for the References section but omit the article title. Conclude the abstract with a summary statement. Because the abstract will be published separately by abstracting services, it must be complete and understandable without reference to the text.

**Introduction.** The introduction should supply sufficient background information to allow the reader to understand and evaluate the results of the present study without referring to previous publications on the topic. The introduction should also provide the hypothesis that was addressed or the rationale for the study. References should be chosen carefully to provide the most salient background rather than an exhaustive review of the topic.

**Case Report.** The Case Report section, placed after the introduction and before Materials and Methods, is optional and gives relevant clinical information about one or more patients.

**Materials and Methods.** The Materials and Methods section should include sufficient technical information to allow the experiments to be repeated. When centrifugation conditions are critical, give enough information to enable another investigator to repeat the procedure: make of centrifuge, model of rotor, temperature, time at maximum speed, and centrifugal force ( $\times g$  rather than revolutions per minute). For commonly used materials and methods (e.g., media and protein concentration determinations), a simple reference is sufficient. If several alternative methods are commonly used, it is helpful to identify the method briefly as well as to cite the reference. For example, it is preferable to state "cells were broken by ultrasonic treatment as previously described (9)" rather than "cells were broken as previously described (9)." This allows the reader to assess the method without constant reference to previous publications. Describe new methods completely, and give sources of unusual chemicals, equipment, or microbial strains. When large numbers of microbial strains or mutants are used in a study, include tables identifying the immediate sources (i.e., sources from whom the strains were obtained) and properties of the strains, mutants, bacteriophages, plasmids, etc.

A method, strain, etc., used in only one of several experiments reported in the paper may be described in the Results section or very briefly (one or two sentences) in a table footnote or figure legend. It is expected that the sources from whom the strains were obtained will be identified.

**Results.** In the Results section, include the rationale or design of the experiments as well as the results; reserve extensive interpretation of the results for the Discussion section. Present the results as concisely as possible in **one** of the following: text, table(s), or figure(s). Avoid extensive use of graphs to present data that might be more concisely or more quantitatively presented in the text or tables. Limit photographs (particularly photomicrographs and electron micrographs) to those that are absolutely necessary to show the experimental findings. Number figures and tables in the order in which they are cited in the text, and be sure that all figures and tables are cited.

**Discussion.** The Discussion should provide an interpretation of the results in relation to previously published work and to the experimental system at hand and should not contain extensive repetition of the Results section or reiteration of the introduction. In short papers, the Results and Discussion sections may be combined.

**Acknowledgments.** The source of any financial support received for the work being published must be indicated in the Acknowledgments section. (It will be

assumed that the absence of such an acknowledgment is a statement by the authors that no support was received.) The usual format is as follows: "This work was supported by Public Health Service grant CA-01234 from the National Cancer Institute."

Recognition of personal assistance should be given as a separate paragraph, as should any statements disclaiming endorsement or approval of the views reflected in the paper or of a product mentioned therein.

**Appendixes.** Appendixes, which contain additional material to aid the reader, are permitted. Titles, authors, and References sections that are distinct from those of the primary article are not allowed. If it is not feasible to list the author(s) of the appendix in the byline or the Acknowledgments section of the primary article, rewrite the appendix so that it can be considered for publication as an independent article, either full-length or Note style. Equations, tables, and figures should be labeled with the letter "A" preceding the numeral to distinguish them from those cited in the main body of the text.

**References. (i) References listed in the References section.** The References section must include all journal articles (both print and online), books and book chapters (both print and online), patents, theses and dissertations, published conference proceedings, meeting abstracts from published abstract books or journal supplements, letters (to the editor), and company publications, as well as in-press journal articles, book chapters, and books (publication title must be given). Arrange the citations in **alphabetical order** (letter by letter, ignoring spaces and punctuation) by first author and **number consecutively**. Provide the names of **all** the authors for each reference. All listed references **must** be cited parenthetically by number in the text. Since title and byline information that is downloaded from PubMed does not show accents, italics, or special characters, authors should refer to the PDF files or hard-copy versions of the articles and incorporate the necessary corrections in the submitted manuscript. Abbreviate journal names according to *BIOSIS Serial Sources* (The Thomson Corporation, Philadelphia, PA, 2006).

Follow the styles shown in the examples below for print references.

1. Arendsen, A. F., M. Q. Solimar, and S. W. Ragsdale. 1999. Nitrate-dependent regulation of acetate biosynthesis and nitrate respiration by *Clostridium thermoaceticum*. *J. Bacteriol.* **181**:1489–1495.
2. Cox, C. S., B. R. Brown, and J. C. Smith. *J. Gen. Genet.*, in press.\* {Article title is optional; journal title is mandatory.}
3. da Costa, M. S., M. F. Nobre, and F. A. Rainey. 2001. Genus I. *Thermus* Brock and Freeze 1969, 295,<sup>AL</sup> emend. Nobre, Trüper and da Costa 1996b, 605, p. 404–414. In D. R. Boone, R. W. Castenholz, and

- G. M. Garrity (ed.), *Bergey's manual of systematic bacteriology*, 2nd ed., vol. 1. Springer, New York, NY.
4. **Elder, B. L., and S. E. Sharp.** 2003. Cumitech 39, Competency assessment in the clinical laboratory. Coordinating ed., S. E. Sharp. ASM Press, Washington, DC.
  5. **Falagas, M. E., and S. K. Kasiakou.** 2006. Use of international units when dosing colistin will help decrease confusion related to various formulations of the drug around the world. *Antimicrob. Agents Chemother.* **50**:2274–2275. (Letter.) {*"Letter" or "Letter to the editor" is allowed but not required at the end of such an entry.*}
  6. **Fitzgerald, G., and D. Shaw.** In A. E. Waters (ed.), *Clinical microbiology*, in press. EFH Publishing Co., Boston, MA.\* {*Chapter title is optional.*}
  7. **Forman, M. S., and A. Valsamakis.** 2003. Specimen collection, transport, and processing: virology, p. 1227–1241. In P. R. Murray, E. J. Baron, M. A. Pfaller, J. H. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. ASM Press, Washington, DC.
  8. **Garcia, C. O., S. Paira, R. Burgos, J. Molina, J. F. Molina, and C. Calvo.** 1996. Detection of salmonella DNA in synovial membrane and synovial fluid from Latin American patients. *Arthritis Rheum.* **39**(Suppl.): S185. {*Meeting abstract published in journal supplement.*}
  9. **Green, P. N., D. Hood, and C. S. Dow.** 1984. Taxonomic status of some methylotrophic bacteria, p. 251–254. In R. L. Crawford and R. S. Hanson (ed.), *Microbial growth on C<sub>1</sub> compounds*. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, DC.
  10. **Odell, J. C.** April 1970. Process for batch culturing. U.S. patent 484,363,770. {*Include the name of the patented item/process if possible; the patent number is mandatory.*}
  11. **O'Malley, D. R.** 1998. Ph.D. thesis. University of California, Los Angeles. {*Title is optional.*}
  12. **Rotimi, V. O., N. O. Salako, E. M. Mohaddas, and L. P. Philip.** 2005. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-1658. {*Abstract title is optional.*}
  13. **Smith, D., C. Johnson, M. Maier, and J. J. Maurer.** 2005. Distribution of fimbrial, phage and plasmid associated virulence genes among poultry *Salmonella enterica* serovars, abstr. P-038, p. 445. Abstr. 105th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC. {*Abstract title is optional.*}
  14. **Stratagene.** 2006. Yeast DNA isolation system: instruction manual. Stratagene, La Jolla, CA. {*Use the company name as the author if none is provided for a company publication.*}

\*A reference to an in-press ASM publication should state the control number (e.g., AAC00577-07) if it is a journal article or the name of the publication if it is a book.

Online references must provide the same information that print references do, but some variation is allowed. For online journal articles, posting or revision dates may replace the year of publication, and a DOI or URL may be provided in addition to or in lieu of volume and page numbers. Some examples follow.

1. **Charlier, D., and N. Glansdorff.** September 2004, posting date. Chapter 3.6.1.10, Biosynthesis of arginine and polyamines. In R. Curtiss III et al. (ed.), *EcoSal—Escherichia coli and Salmonella: cellular and molecular biology*. ASM Press, Washington, DC. <http://www.ecosal.org>. {*Note that each chapter has its own posting date.*}
2. **Dionne, M. S., and D. S. Schneider.** 2002. Screening the fruitfly immune system. *Genome Biol.* **3**: REVIEWS1010. <http://genomebiology.com/2002/3/4/reviews/1010>.
3. **Smith, F. X., H. J. Merianos, A. T. Brunger, and D. M. Engelman.** 2001. Polar residues drive association of polyleucine transmembrane helices. *Proc. Natl. Acad. Sci. USA* **98**:2250–2255. doi:10.1073/pnas.041593698.
4. **Winnick, S., D. O. Lucas, A. L. Hartman, and D. Toll.** 2005. How do you improve compliance? *Pediatrics* **115**:e718–e724.

NOTE: A posting or accession date is required for any online reference that is periodically updated or changed.

(ii) **References cited in the text.** References to unpublished data, manuscripts submitted for publication, unpublished conference presentations (e.g., a report or poster that has not appeared in published conference proceedings), personal communications, patent applications and patents pending, computer software, databases, and websites (home pages) should be made parenthetically in the text as follows.

... similar results (R. B. Layton and C. C. Weathers, unpublished data).

... system was used (J. L. McInerney, A. F. Holden, and P. N. Brighton, submitted for publication).

... as described previously (M. G. Gordon and F. L. Rattner, presented at the Fourth Symposium on Food Microbiology, Overton, IL, 13 to 15 June 1989). {*For nonpublished abstracts, posters, etc.*}

... this new process (V. R. Smoll, 20 June 1999, Australian Patent Office). {*For non-U.S. patent applications, give the date of publication of the application.*}

... available in the GenBank database (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>).

... using ABC software (version 2.2; Department of Microbiology, State University [<http://www.stu.micro>]).

**URLs for companies that produce any of the products mentioned in your study or for products being sold may**

**NOT be included in the article. However, company URLs that permit access to scientific data related to the study or to shareware used in the study are permitted.**

**(iii) References related to supplemental material.** References that are related **only** to supplemental material hosted by ASM or posted on a personal/institutional website should not be listed in the References section of an article; include them with the supplemental material itself.

**(iv) Referencing publish-ahead-of-print manuscripts.** Citations of ASM Accepts manuscripts should look like the following example.

**Wang, G. G., M. P. Pasillas, and M. P. Kamps.** 15 May 2006. Persistent transactivation by Meis1 replaces Hox function in myeloid leukemogenesis models: evidence for co-occupancy of Meis1-Pbx and Hox-Pbx complexes on promoters of leukemia-associated genes. *Mol. Cell. Biol.* doi:10.1128/MCB.00586-06.

If an author of an article cites an ASM Accepts manuscript in his paper but wishes at the proof stage to change the reference entry to that for the published article, the following style should be used:

**Wang, G. G., M. P. Pasillas, and M. P. Kamps.** 15 May 2006. Persistent transactivation by Meis1 replaces Hox function in myeloid leukemogenesis models: evidence for co-occupancy of Meis1-Pbx and Hox-Pbx complexes on promoters of leukemia-associated genes. *Mol. Cell. Biol.* doi:10.1128/MCB.00586-06. (Subsequently published, *Mol. Cell. Biol.* **26**:3902–3916, 2006.)

Other journals may use different styles for their publish-ahead-of-print manuscripts, but citation entries must include the following information: author name(s), posting date, title, journal title, and volume and page numbers and/or DOI. The following is an example:

**Zhou, F. X., H. J. Merianos, A. T. Brunger, and D. M. Engelman.** 13 February 2001, posting date. Polar residues drive association of polyleucine transmembrane helices. *Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.041593698.

## Notes

The Note format is intended for the presentation of brief observations that do not warrant full-length papers. Submit Notes in the same way as full-length papers. *They receive the same review, they are not published more rapidly than full-length papers, and they are not considered preliminary communications.*

Each Note must have an **abstract of no more than 50 words**. Do not use section headings in the body of the Note; combine methods, results, and discussion in a single section. Paragraph lead-ins are permissible. The text

should be kept to a minimum and if possible **should not exceed 1,000 words**; the number of figures and tables should also be kept to a minimum. **Materials and methods should be described in the text, not in figure legends or table footnotes.** Present acknowledgments as in full-length papers, but do not use a heading. The References section is identical to that of full-length papers.

## Minireviews

Minireviews are brief (**limit of 6 printed pages exclusive of references**) biographical profiles, historical perspectives, or summaries of developments in fast-moving areas of chemotherapy. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of AAC. For example, subject matter may range from structure-activity correlates among a group of semisynthetic cephalosporins to the comparative efficacies of new and old drugs in the prevention or treatment of diseases of microbial origin in humans.

Minireviews may be either solicited or proffered by authors responding to a recognized need. Irrespective of origin, Minireviews are subject to review and should be submitted via Rapid Review. The cover letter should state whether the article was solicited and by whom.

Minireviews do not have abstracts. In the Abstract section of the submission form, put "Not Applicable." The body of the Minireview may either have section headings or be set up like a Note (see above).

## Guest Commentaries

Guest Commentaries are *invited* communications concerning relevant topics within the scope of this journal that are not necessarily covered by Minireviews. They are intended to engender discussion and stimulate consensus statements by such organizations as the American Academy of Microbiology, Clinical and Laboratory Standards Institute, etc. Reviews of the literature, methods and other how-to papers, and responses targeted at a specific published paper are not appropriate. Guest Commentaries are subject to review.

The length may not exceed 4 printed pages, and the format is like that of a Minireview (see above). Commentaries should be submitted via Rapid Review.

## Letters to the Editor

Two types of Letters to the Editor may be submitted. The first type (Comment Letter) is intended for comments on articles published previously in the journal and must cite published references to support the writer's argument. The second type (New-Data Letter) may report new, concise findings that are not appropriate for publication as full-length papers or Notes.

Letters may be **no more than 500 words long and must be typed double spaced**. Refer to a recently published Letter for correct formatting. Note that authors and affiliations are listed at the foot of the Letter. Provide only the primary affiliation for each author.

All Letters to the Editor must be submitted electronically, and the type of Letter (New Data or Comment) must be selected from the drop-down list in the submission form. For Letters commenting on published articles, the cover letter should state the volume and issue in which the article was published, the title of the article, and the last name of the first author. In the Abstract section of the submission form, put "Not Applicable." Letters to the Editor do not have abstracts. Both types of Letter must have a title, which must appear on the manuscript and on the submission form. Figures and tables should be kept to a minimum.

If the Letter is related to a published article, it will be sent to the editor who handled the article in question. If the editor believes that publication is warranted, he will solicit a reply from the corresponding author of the article and give approval for publication.

New-Data Letters will be assigned to an editor according to subject matter and will be reviewed by that editor and/or a reviewer.

**Please note that some indexing/abstracting services do not include Letters to the Editor in their databases.**

### Errata

The Erratum section provides a means of correcting errors that occurred during the writing, typing, editing, or printing (e.g., a misspelling, a dropped word or line, or mislabeling in a figure) of a published article. Submit Errata via Rapid Review (see "How To Submit Manuscripts," above). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Erratum as an MS Word file. Please see a recent issue for correct formatting.

### Authors' Corrections

The Author's Correction section provides a means of correcting errors of omission (e.g., author names or citations) and errors of a scientific nature that do not alter the overall basic results or conclusions of a published article (e.g., an incorrect unit of measurement or order of magnitude used throughout, contamination of one of numerous cultures, or misidentification of a mutant strain, causing erroneous data for only a portion [noncritical] of the study). *Note that the addition of new data is not permitted.*

For corrections of a scientific nature or issues involving authorship, including contributions and use or ownership of data and/or materials, all disputing parties must agree, in writing, to publication of the Correction. For omission of an author's name, letters must be signed by the authors of the article and the author whose name was omitted. The editor who handled the article will be consulted if necessary.

Submit an Author's Correction via Rapid Review (see "How To Submit Manuscripts," above). In the submission form, select Erratum as the manuscript type; there is no separate selection in Rapid Review for Authors' Corrections, but your Correction will be published as such if appropriate. In the Abstract section of the submission form (a required field), put "Not Applicable."

Upload the text of your Author's Correction as an MS Word file. Please see a recent issue for correct formatting. Signed letters of agreement must be supplied as supplemental material (scanned PDF files).

### Retractions

Retractions are reserved for major errors or breaches of ethics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Submit Retractions via Rapid Review (see "How To Submit Manuscripts," above). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Retraction as an MS Word file. Letters of agreement signed by all of the authors must be supplied as supplemental material (scanned PDF files). The Retraction will be assigned to the editor in chief of the journal, and the editor who handled the paper and the chairman of the ASM Publication Board will be consulted. If all parties agree to the publication and content of the Retraction, it will be sent to the Journals Department for publication.

### ILLUSTRATIONS AND TABLES

**Digital files that are acceptable for production (see below) must be provided for all illustrations on return of the modified manuscript. (On initial submission, the entire paper may be submitted in PDF format.)**

We strongly recommend that before returning their modified manuscripts, authors check the acceptability of their digital images for production by running their files through Rapid Inspector, a tool provided at the following URL: <http://rapidinspector.cadmus.com/mw/>. Rapid Inspector is an easy-to-use Web-based application that identifies file characteristics that may render the

Application	Macintosh	
	File type	
	Black and white	Color (CMYK) <sup>a</sup>
Adobe Illustrator 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 CS	EPS	EPS
Adobe InDesign 1.0	EPS	EPS
Adobe PageMaker 6.5	EPS	EPS
Adobe Photoshop 4.0, 5.0, 5.5, 6.0, 7.0, 8.0 CS	TIFF	TIFF
Adobe Photoshop 5.0 LE	TIFF	N/A <sup>b</sup>
ChemDraw Pro 5.0	EPS/TIFF	EPS/TIFF
Corel Photo-Paint 8.0	TIFF	EPS
CorelDRAW 6.0, 8.0	EPS/TIFF	EPS
Deneba Canvas 6.0, 7.0, 8.0	EPS/TIFF	EPS
Macromedia FreeHand 7.0, 8.0, 9.0	EPS	EPS
PowerPoint 98, 2001	PPT <sup>c</sup>	N/A <sup>b</sup>
Prism 3 by GraphPad	TIFF	N/A <sup>b</sup>
Synergy Kaleidagraph 3.08, 3.51	EPS	N/A <sup>b</sup>

<sup>a</sup>Color graphics must be saved and printed in the CMYK mode, not RGB.

<sup>b</sup>ASM accepts only black-and-white, not color, graphics created with Kaleidagraph, Adobe Photoshop 5.0 LE, Prism 3 by GraphPad, and PowerPoint.

<sup>c</sup>For instructions on saving PowerPoint files, refer to the Cadmus digital art website at <http://cjs.cadmus.com/da/index.jsp>.

## Windows

Application	File type	
	Black and white	Color (CMYK) <sup>a</sup>
Adobe Illustrator 7.0, 8.0, 9.0, 10.0, 11.0 CS	EPS	EPS
Adobe InDesign 1.0	EPS	EPS
Adobe PageMaker 6.5	EPS	EPS
Adobe Photoshop 4.0, 5.0, 5.5, 6.0, 7.0, 8.0 CS	TIFF	TIFF
Adobe Photoshop 5.0 LE	TIFF	N/A <sup>b</sup>
ChemDraw Pro 5.0	EPS/TIFF	EPS/TIFF
Corel Photo-Paint 8.0, 9.0	TIFF	EPS
CorelDRAW 7.0, 8.0, 9.0	EPS/TIFF	EPS
Deneba Canvas 6.0, 7.0	EPS/TIFF	EPS
Macromedia FreeHand 7.0, 8.0, 9.0	EPS	EPS
PowerPoint 97, 2000, XP	PPT <sup>c</sup>	N/A <sup>b</sup>
Prism 3 by GraphPad	TIFF	N/A <sup>b</sup>
SigmaPlot 8.01	EPS	EPS

<sup>a</sup>Color graphics must be saved and printed in the CMYK mode, *not* RGB.

<sup>b</sup>ASM accepts only black-and-white, not color, graphics created with Adobe Photoshop 5.0 LE, Prism 3 by GraphPad, and PowerPoint.

<sup>c</sup>For instructions on saving PowerPoint files, refer to the Cadmus digital art website at <http://cjs.cadmus.com/da/index.jsp>.

image unusable for production.

Illustrations may be continuous-tone images, line drawings, or composites. Color graphics may be submitted, but the cost of printing in color must be borne by the author. Suggestions about how to reduce costs and ensure accurate color reproduction are given below.

The preferred format for tables is MS Word; however, WordPerfect and Acrobat PDF are also acceptable (see the section on Tables below).

## Image Manipulation

Computer-generated images may be processed only minimally. Processing (e.g., changing contrast, brightness, or color balance) is acceptable only if applied to all parts of the image, as well as to the controls, equally, and descriptions of all such adjustments and the tools used (both hardware and software) must be provided in the manuscript. Unprocessed data and files must be retained by the authors and be provided to the editor on request.

## Illustrations

**File types and formats.** As mentioned above, illustrations may be supplied as PDF files for reviewing purposes only on initial submission; in fact, we recommend this option to minimize file upload time. At the modification stage, production quality digital files must be submitted: TIFF or EPS files from supported applications or PowerPoint files (black and white only). Except for figures produced in PowerPoint, all graphics submitted with modified manuscripts must be bitmap, grayscale, or CMYK (*not* RGB). Halftone images (those with various densities or shades) must be grayscale, *not* bitmap.

Color PowerPoint files are *not* accepted because the application, designed for developing on-screen computer presentations, uses the RGB color mode whereas

the printing process uses the CMYK color mode. Colors that are represented in a PowerPoint image may not be reproducible on a printing press. Although black-and-white Microsoft PowerPoint files are accepted, we do *not* recommend the use of PowerPoint. PowerPoint requires users to pay close attention to the fonts used in their images (see the section on Fonts below). If instructions for fonts are not followed *exactly*, images prepared for publication are subject to missing characters, improperly converted characters, or shifting/obscuring of elements or text in the figure. *Use of PowerPoint is therefore not recommended for either color or black-and-white illustrations.*

Acceptable file types and formats for production are given in the charts above. More-detailed instructions for preparing illustrations are available at <http://cjs.cadmus.com/da>. Please review this information before preparing your files. If you require additional information, please send an e-mail inquiry to [digitalart@cadmus.com](mailto:digitalart@cadmus.com).

**Minimum resolution.** It is extremely important that a high enough resolution is used. Any imported images must be at the correct resolution before they are placed. Note, however, that the higher the resolution, the larger the file and the longer the upload time. Publication quality will *not* be improved by using a resolution higher than the minimum. Minimum resolutions are as follows:

- 300 dpi for grayscale and color
- 600 dpi for lettering
- 1,200 dpi for line art
- 600 dpi for combination art (lettering and images)

**Size.** All graphics **MUST** be submitted at their **intended publication size**; that is, the image uploaded should be 100% of its print dimensions so that no reduction or enlargement is necessary. Resolution must be at the required level at the submitted size. Include only the significant portion of an illustration. White space must be cropped from the image, and excess space between panel labels and the image must be eliminated.

- Maximum width for a 1-column figure: 3<sup>5</sup>/<sub>16</sub> inches (ca. 8.4 cm)
- Maximum width for a 2-column figure: 6<sup>7</sup>/<sub>8</sub> inches (ca. 17.4 cm)
- Minimum width for a 2-column figure: 4<sup>1</sup>/<sub>4</sub> inches (10.8 cm)
- Maximum height: 9<sup>1</sup>/<sub>16</sub> inches (23.0 cm)

**Contrast.** Illustrations must contain sufficient contrast to withstand the inevitable loss of contrast and detail inherent in the printing process. See also the section on color illustrations below.

**Labeling and assembly.** All final lettering, labeling, tooling, etc., **MUST** be incorporated into the figures. It cannot be added at a later date. If a figure number is included, it **must** appear well outside the boundaries of

the image itself. (Numbering may need to be changed at the copyediting stage.) Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file; i.e., rather than uploading a separate file for each panel in a figure, assemble all panels in one piece and supply them as one file.

**Fonts.** To avoid font problems, set all type in one of the following fonts: Helvetica, Times Roman, European PI, Mathematical PI, or Symbol. All fonts other than these five must be converted to paths (or outlines) in the application with which they were created. For proper font use in PowerPoint images, refer to the Cadmus digital art website, [http://cjs.cadmus.com/da/instructions/ppt\\_disclaimer.jsp](http://cjs.cadmus.com/da/instructions/ppt_disclaimer.jsp).

**Compression.** Images created with Macintosh applications may be compressed with Stuffit. Images created with Windows applications may be compressed with WINZIP or PKZIP.

**Color illustrations.** *The cost of printing in color must be borne by the author.* The current color cost per figure may be accessed from the submission form in Rapid Review. For accepted manuscripts, the total cost of the color will be included in the acceptance letter sent out by ASM. Adherence to the following guidelines, in addition to the general ones below, will help to minimize costs and to ensure color reproduction that is as accurate as possible.

Because of the requirements of print production, color illustrations **must** be in the CMYK (cyan, magenta, yellow, black) color space. The "normal" color mode for most computer software is RGB (red, green, blue), which is also the color space of your computer monitor. Since CMYK is a smaller color space (meaning it can define fewer colors), colors often shift when an RGB file is converted to CMYK. In particular, figures showing red or green fluorescence and those with a significant range of colors may be difficult or impossible to reproduce during the printing process.

Color illustrations must be supplied in the CMYK color mode, as either (i) CMYK TIFF images with a resolution of at least 300 pixels per inch (raster files, consisting of pixels) or (ii) Illustrator-compatible EPS files with CMYK color elements (vector files, consisting of lines, fonts, fills, and images). See the charts above for a list of supported applications.

We cannot accept any Microsoft Office files (PowerPoint, Word, Excel) for color illustrations because they are restricted to the RGB color space.

## Drawings

Submit graphs, charts, complicated chemical or mathematical formulas, diagrams, and other drawings as finished products not requiring additional artwork or typesetting. No part of the graph or drawing may be handwritten. *All* elements, including letters, numbers,

and symbols, *must* be easily readable, and both axes of a graph must be labeled. Keep in mind that the journal is published both in print and online and that the same electronic files submitted by the authors are used to produce both.

When creating line art, please use the following guidelines:

1. **All art MUST be submitted at its intended publication size.** For acceptable dimensions, see the Size section above.
2. **Avoid using screens (i.e., shading)** in line art. It can be difficult and time-consuming to reproduce these images without moiré patterns. Various pattern backgrounds are preferable to screens as long as the patterns are not imported from another application. If you must use images containing screens,
  - Generate the image at line screens of 85 lines per inch or lower.
  - When applying multiple shades of gray, differentiate the gray levels by at least 20%.
  - Never use levels of gray below 20% or above 70% as they will fade out or become totally black upon scanning and reduction.
3. Use thick, solid lines that are no finer than 1 point in thickness.
4. No type should be smaller than 6 points at the final publication size.
5. Avoid layering type directly over shaded or textured areas.
6. Avoid the use of reversed type (white lettering on a black background).
7. Avoid heavy letters, which tend to close up, and unusual symbols, which the printer may not be able to reproduce in the legend.
8. If colors are used, avoid using similar shades of the same color and avoid very light colors.

In figure ordinate and abscissa scales (as well as table column headings), **avoid the ambiguous use of numbers with exponents.** Usually, it is preferable to use the *Système International d'Unités* (SI) symbols ( $\mu$  for  $10^{-6}$ , m for  $10^{-3}$ , k for  $10^3$ , M for  $10^6$ , etc.). A complete listing of SI symbols can be found in the International Union of Pure and Applied Chemistry (IUPAC) publication *Quantities, Units and Symbols in Physical Chemistry* (Blackwell Science, Oxford, United Kingdom, 1993); an abbreviated list is available at <http://www.iupac.org/reports/1993/homann/index.html>. Thus, a representation of 20,000 cpm on a figure ordinate should be made by the number 20 accompanied by the label kcpm.

When powers of 10 must be used, the journal requires that the exponent power be associated with the number shown. In representing 20,000 cells per ml, the numeral on the ordinate would be "2" and the label would be "10<sup>4</sup> cells per ml" (not "cells per ml  $\times 10^{-4}$ "). Likewise, an enzyme activity of 0.06 U/ml would be shown as 6



accompanied by the label " $10^{-2}$  U/ml." The preferred designation would be 60 mU/ml (milliunits per milliliter).

### Presentation of Nucleic Acid Sequences

Nucleic acid sequences of limited length which are the primary subject of a study may be presented freestyle in the most effective format. Longer nucleic acid sequences must be presented as figures in the following format to conserve space. Print the sequence in lines of approximately 100 to 120 nucleotides in a nonproportional (monospace) font that is easily legible when published with a line length of 6 inches (ca. 15.2 cm). If possible, lines of nucleic acid sequence should be further subdivided into blocks of 10 or 20 nucleotides by spaces within the sequence or by marks above it. Uppercase and lowercase letters may be used to designate the exon-intron structure, transcribed regions, etc., if the lowercase letters remain legible at a 6-inch (ca. 15.2-cm) line length. Number the sequence line by line; place numerals, representing the first base of each line, to the left of the lines. **Minimize spacing between lines of sequence, leaving room only for annotation of the sequence.** Annotation may include boldface, underlining, brackets, boxes, etc. Encoded amino acid sequences may be presented, if necessary, immediately above or below the first nucleotide of each codon, by using the single-letter amino acid symbols. Comparisons of multiple nucleic acid sequences should conform as nearly as possible to the same format.

### Figure Legends

Legends should provide enough information so that the figure is understandable without frequent reference to the text. However, detailed experimental methods must be described in the Materials and Methods section, not in a figure legend. A method that is unique to one of several experiments may be set forth in a legend only if the description is very brief (one or two sentences). Define all symbols used in the figure and define all abbreviations that are not used in the text.

### Tables

Tables that contain artwork, chemical structures, or shading must be submitted as illustrations in an acceptable format at the modification stage. The preferred format for regular tables is MS Word; however, WordPerfect and Acrobat PDF are also acceptable. Note that a straight Excel file is *not* currently an acceptable format. Excel files must be either embedded in a Word or WordPerfect document or converted to PDF *before* being uploaded. **If your modified manuscript contains PDF tables, select "for reviewing purposes only" at the beginning of the file upload process.**

Tables should be formatted as follows. Arrange the data so that **columns of like material read down, not across.** The headings should be sufficiently clear so that the meaning of the data is understandable without reference to the text. See the Abbreviations section (p. 19)

TABLE 1. Distribution of protein and ATPase in fractions of dialyzed membranes<sup>a</sup>

Membrane	Fraction	ATPase	
		U/mg of protein	Total U
Control	Depleted membrane	0.036	2.3
	Concentrated supernatant	0.134	4.82
E1 treated	Depleted membrane	0.034	1.98
	Concentrated supernatant	0.11	4.6

<sup>a</sup> Specific activities of ATPase of nondepleted membranes from control and treated bacteria were 0.21 and 0.20, respectively.

of these Instructions for those that should be used in tables. Explanatory footnotes are acceptable, but more extensive table "legends" are not. Footnotes should not include detailed descriptions of the experiment. Tables must include enough information to warrant table format; those with fewer than six pieces of data will be incorporated into the text by the copy editor. Table 1 is an example of a well-constructed table.

Avoid tables (or figures) of raw data on drug susceptibility, therapeutic activity, or toxicity. Such data should be analyzed by an approved procedure, and the results should be presented in tabular form.

### NOMENCLATURE

#### Chemical and Biochemical Nomenclature

The recognized authority for the names of chemical compounds is *Chemical Abstracts* (CAS, Columbus, OH) and its indexes. *The Merck Index*, 14th ed. (Merck & Co., Inc., Whitehouse Station, NJ, 2006), is also an excellent source. For guidelines to the use of biochemical terminology, consult *Biochemical Nomenclature and Related Documents* (Portland Press, London, United Kingdom, 1992), available at <http://www.chem.qmul.ac.uk/iupac/bibliog/white.html>, and the instructions to authors of the *Journal of Biological Chemistry* and the *Archives of Biochemistry and Biophysics* (first issues of each year).

Molecular weight should not be expressed in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name as assigned by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in *Enzyme Nomenclature* (Academic Press, Inc., New York, NY, 1992) and at <http://www.chem.qmul.ac.uk/iubmb/enzyme/>. If a nonrecommended name is used, place the proper (trivial) name in parentheses at first use in the abstract and text. Use the EC number when one has been assigned, and express enzyme activity either in katal (preferred) or in the older system of micromoles per minute.

#### Nomenclature of Microorganisms

Binary names, consisting of a generic name and a specific epithet (e.g., *Escherichia coli*), must be used for



all microorganisms. Names of categories at or above the genus level may be used alone, but specific and subspecific epithets may not. A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Names of all taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be italicized (or underlined) in the manuscript; strain designations and numbers are not. Vernacular (common) names should be in lowercase roman type (e.g., streptococcus, brucella). For *Salmonella*, genus, species, and subspecies names should be rendered in standard form: *Salmonella enterica* at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. *arizonae* at first use, *S. enterica* subsp. *arizonae* thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar Typhimurium. After the first use, the serovar may also be given without a species name: *Salmonella* serovar Typhimurium. For other information regarding serovar designations, see *Antigenic Formulas of the Salmonella Serovars*, 8th ed. (M. Y. Popoff, WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France, 2001). For a summary of the current standards for *Salmonella* nomenclature and the Kaufmann-White criteria, see the article by Brenner et al. (J. Clin. Microbiol. 38:2465–2467, 2000), the opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes (Int. J. Syst. Evol. Microbiol. 55:519–520, 2005), and the article by Tindall et al. (Int. J. Syst. Evol. Microbiol. 55:521–524, 2005).

The spelling of bacterial names should follow the *Approved Lists of Bacterial Names (Amended) & Index of the Bacterial and Yeast Nomenclatural Changes* (V. B. D. Skerman et al., ed., ASM Press, Washington, DC, 1989) and the validation lists and notification lists published in the *International Journal of Systematic and Evolutionary Microbiology* (formerly the *International Journal of Systematic Bacteriology*) since January 1989. In addition, two sites on the World Wide Web list current approved bacterial names: Bacterial Nomenclature Up-to-Date ([http://www.dsmz.de/microorganisms/main.php?contentleft\\_id=14](http://www.dsmz.de/microorganisms/main.php?contentleft_id=14)) and List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.cict.fr>). If there is reason to use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title and at its first use in the abstract and the text and an appropriate statement concerning the nomenclatural status of the name should be made in the text. "*Candidatus*" species should always be set in quotation marks.

Since the classification of fungi is not complete, it is the responsibility of the author to determine the accepted binomial for a given organism. Sources for these names include *The Yeasts: a Taxonomic Study*, 4th ed. (C. P. Kurtzman and J. W. Fell, ed., Elsevier Science Publishers B.V., Amsterdam, The Netherlands, 1998), and *Ainsworth and Bisby's Dictionary of the Fungi*, 9th ed.

(P. M. Kirk, P. F. Cannon, J. C. David, and J. A. Stalpers, ed., CABI Publishing, Wallingford, Oxfordshire, United Kingdom, 2001); see also <http://www.speciesfungorum.org/Names/Fundic.asp>.

Names used for viruses should be those approved by the International Committee on Taxonomy of Viruses (ICTV) and published in *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses* (C. M. Fauquet et al., ed., Elsevier Academic Press, San Diego, CA, 2005). In addition, the recommendations of the ICTV regarding the use of species names should generally be followed: when the entire species is discussed as a taxonomic entity, the species name, like other taxa, is italic and has the first letter and any proper nouns capitalized (e.g., *Tobacco mosaic virus*, *Murray Valley encephalitis virus*). When the behavior or manipulation of individual viruses is discussed, the vernacular (e.g., tobacco mosaic virus, Murray Valley encephalitis virus) should be used. If desired, synonyms may be added parenthetically when the name is first mentioned. Approved generic (or group) and family names may also be used.

Microorganisms, viruses, and plasmids should be given designations consisting of letters and serial numbers. It is generally advisable to include a worker's initials or a descriptive symbol of locale, laboratory, etc., in the designation. Each new strain, mutant, isolate, or derivative should be given a new (serial) designation. This designation should be distinct from those of the genotype and phenotype, and genotypic and phenotypic symbols should not be included. Plasmids are named with a lowercase "p" followed by the designation in uppercase letters and numbers. To avoid the use of the same designation as that of a widely used strain or plasmid, check the designation against a publication database such as Medline.

## Genetic Nomenclature

To facilitate accurate communication, **it is important that standard genetic nomenclature be used whenever possible and that deviations or proposals for new naming systems be endorsed by an appropriate authoritative body.** Review and/or publication of submitted manuscripts that contain new or nonstandard nomenclature may be delayed by the editor or the Journals Department so that they may be reviewed by the Genetics and Genomics Committee of the ASM Publications Board.

**Before submission of manuscripts, authors may direct questions on genetic nomenclature to the committee's chairman: Maria Costanzo (e-mail: [maria@genome.stanford.edu](mailto:maria@genome.stanford.edu)).** Such a consultation should be mentioned in the manuscript submission letter.

**Bacteria.** The genetic properties of bacteria are described in terms of phenotypes and genotypes. The phenotype describes the observable properties of an organism. The genotype refers to the genetic constitution of an organism, usually in reference to some standard wild type. The guidelines that follow are based on the recommendations of Demerec et al. (Genetics 54:61–76, 1966).

(i) Phenotype designations must be used when mutant loci have not been identified or mapped. They can also be used to identify the protein product of a gene, e.g., the OmpA protein. Phenotype designations generally consist of three-letter symbols; these are *not* italicized, and the first letter of the symbol is capitalized. It is preferable to use Roman or Arabic numerals (instead of letters) to identify a series of related phenotypes. Thus, a series of nucleic acid polymerase mutants might be designated Pol1, Pol2, Pol3, etc. Wild-type characteristics can be designated with a superscript plus (Pol<sup>+</sup>), and, when necessary for clarity, negative superscripts (Pol<sup>-</sup>) can be used to designate mutant characteristics. Lowercase superscript letters may be used to further delineate phenotypes (e.g., Str<sup>r</sup> for streptomycin resistance). Phenotype designations should be defined.

(ii) Genotype designations are also indicated by three-letter locus symbols. In contrast to phenotype designations, these are lowercase italic (e.g., *ara his rps*). If several loci govern related functions, these are distinguished by italicized capital letters following the locus symbol (e.g., *araA araB araC*). Promoter, terminator, and operator sites should be indicated as described by Bachmann and Low (Microbiol. Rev. 44:1-56, 1980): e.g., *lacZp*, *lacAt*, and *lacZo*.

(iii) Wild-type alleles are indicated with a superscript plus (*ara*<sup>+</sup> *his*<sup>+</sup>). A superscript minus is not used to indicate a mutant locus; thus, one refers to an *ara* mutant rather than an *ara*<sup>-</sup> strain.

(iv) Mutation sites are designated by placing serial isolation numbers (allele numbers) after the locus symbol (e.g., *araA1 araA2*). If only a single such locus exists or if it is not known in which of several related loci the mutation has occurred, a hyphen is used instead of the capital letter (e.g., *ara-23*). It is essential in papers reporting the isolation of new mutants that allele numbers be given to the mutations. For *Escherichia coli*, there is a registry of such numbers: *E. coli* Genetic Stock Center, Department of Biology, Yale University, New Haven, CT 06511-5188. For the genus *Salmonella*, the registry is *Salmonella* Genetic Stock Center, Department of Biology, University of Calgary, Calgary, Alberta T2N 1N4, Canada. For the genus *Bacillus*, the registry is *Bacillus* Genetic Stock Center, Ohio State University, Columbus, OH 43210.

(v) The use of superscripts with genotypes (other than + to indicate wild-type alleles) should be avoided. Designations indicating amber mutations (Am), temperature-sensitive mutations (Ts), constitutive mutations (Con), cold-sensitive mutations (Cs), production of a hybrid protein (Hyb), and other important phenotypic properties should follow the allele number [e.g., *araA230*(Am) *hisD21*(Ts)]. All other such designations of phenotype must be defined at the first occurrence. If superscripts must be used, they must be approved by the editor and defined at the first occurrence in the text.

Subscripts may be used in two situations. Subscripts may be used to distinguish between genes (having the same name) from different organisms or strains, e.g.,

*his*<sub>*E. coli*</sub> or *his*<sub>K-12</sub> for the *his* genes of *E. coli* or strain K-12 in another species or strain, respectively. An abbreviation may also be used if it is explained. Similarly, a subscript is also used to distinguish between genetic elements that have the same name. For example, the promoters of the *gln* operon can be designated *glnAp*<sub>1</sub> and *glnAp*<sub>2</sub>. This form departs slightly from that recommended by Bachmann and Low (e.g., *desC1p*).

(vi) Deletions are indicated by the symbol Δ placed before the deleted gene or region, e.g., Δ*trpA432*, Δ(*aroP-aceE*)<sub>419</sub>, or Δ*his*(*dhuA hisJ hisQ*)<sub>1256</sub>. Similarly, other symbols can be used (with appropriate definition). Thus, a fusion of the *ara* and *lac* operons can be shown as Φ(*ara-lac*)<sub>95</sub>. Likewise, Φ(*araB'-lacZ*)<sub>96</sub> indicates that the fusion results in a truncated *araB* gene fused to an intact *lacZ* gene, and Φ(*malE-lacZ*)<sub>97</sub>(Hyb) shows that a hybrid protein is synthesized. An inversion is shown as IN(*rmD-rmE*)<sub>1</sub>. An insertion of an *E. coli his* gene into plasmid pSC101 at zero kilobases (0 kb) is shown as pSC101 Ω(0kb::K-12*hisB*)<sub>4</sub>. An alternative designation of an insertion can be used in simple cases, e.g., *galT236::Tn5*. The number 236 refers to the locus of the insertion, and if the strain carries an additional *gal* mutation, it is listed separately. Additional examples, which utilize a slightly different format, can be found in the papers by Campbell et al. and Novick et al. cited below. It is important in reporting the construction of strains in which a mobile element was inserted and subsequently deleted that this fact be noted in the strain table. This can be done by listing the genotype of the strain used as an intermediate in a table footnote or by making a direct or parenthetical remark in the genotype, e.g., (F<sup>-</sup>), Δ*Mu* cts, or *mal::ΔMu* cts:*lac*. In setting parenthetical remarks within the genotype or dividing the genotype into constituent elements, parentheses and brackets are used without special meaning; brackets are used outside parentheses. To indicate the presence of an episome, parentheses (or brackets) are used (λ, F<sup>+</sup>). Reference to an integrated episome is indicated as described above for inserted elements, and an exogenote is shown as, for example, W3110/F'<sub>8</sub>(*gal*<sup>+</sup>).

For information about genetic maps of locus symbols in current use, consult Berlyn (Microbiol. Mol. Biol. Rev. 62:814-984, 1998) for *E. coli* K-12, Sanderson and Roth (Microbiol. Rev. 52:485-532, 1988) for *Salmonella* serovar Typhimurium, Holloway et al. (Microbiol. Rev. 43:73-102, 1979) for the genus *Pseudomonas*, Piggot and Hoch (Microbiol. Rev. 49:158-179, 1985) for *Bacillus subtilis*, Perkins et al. (Microbiol. Rev. 46:426-570, 1982) for *Neurospora crassa*, and Mortimer and Schild (Microbiol. Rev. 49:181-213, 1985) for *Saccharomyces cerevisiae*. For yeasts, *Chlamydomonas* spp., and several fungal species, symbols such as those given in the *Handbook of Microbiology*, 2nd ed. (A. I. Laskin and H. A. Lechevalier, ed., CRC Press, Inc., Cleveland, OH, 1988) should be used.

**Conventions for naming genes.** It is recommended that (entirely) new genes be given names that are mnemonic-

ics of their function, avoiding names that are already assigned and earlier or alternative gene names, irrespective of the bacterium for which such assignments have been made. Similarly, it is recommended that, whenever possible, homologous genes present in different organisms receive the same name. When homology is not apparent or the function of a new gene has not been established, a provisional name may be given by one of the following methods. (i) The gene may be named on the basis of its map location in the style *yaaA*, analogous to the style used for recording transposon insertions (*zef*) as discussed below. A list of such names in use for *E. coli* has been published by Rudd (Microbiol. Mol. Biol. Rev. 62:985–1019, 1998). (ii) A provisional name may be given in the style described by Demerec et al. (e.g., *usg*, gene upstream of *folC*). Such names should be unique, and names such as *orf* or *genX* should not be used. For reference, the *E. coli* Genetic Stock Center's database includes an updated listing of *E. coli* gene names and gene products. It is accessible on the Internet (<http://cgsc.biology.yale.edu/cgsc.html>). The Center's relational database can also be searched via Telnet; for access, send a request to [berlyn@cgsc.biology.yale.edu](mailto:berlyn@cgsc.biology.yale.edu). A list can also be found in the work of Riley (Microbiol. Rev. 57:862–952, 1993). For the genes of other bacteria, consult the references given above.

**“Mutant” versus “mutation.”** Keep in mind the distinction between a *mutation* (an alteration of the primary sequence of the genetic material) and a *mutant* (a strain carrying one or more mutations). One may speak about the mapping of a mutation, but one cannot map a mutant. Likewise, a mutant has no genetic locus, only a phenotype.

**“Homology” versus “similarity.”** For use of terms that describe relationships between genes, consult the articles by Theissen (Nature 415:741, 2002) and Fitch (Trends Genet. 16:227–231, 2000). “Homology” implies a relationship between genes that share a common evolutionary origin; partial homology is not recognized. When sequence comparisons are discussed, it is more appropriate to use the term “percent sequence similarity” or “percent sequence identity,” as appropriate.

**Strain designations.** Do not use a genotype as a name (e.g., “. . . subsequent use of *leuC6* for transduction. . .”). If a strain designation has not been chosen, select an appropriate word combination (e.g., “another strain containing the *leuC6* mutation”).

**Viruses.** The genetic nomenclature for viruses differs from that for bacteria. In most instances, viruses have no phenotype, since they have no metabolism outside host cells. Therefore, distinctions between phenotype and genotype cannot be made. Superscripts are used to indicate hybrid genomes. Genetic symbols may be one, two, or three letters. For example, a mutant strain of  $\lambda$  might be designated  $\lambda$  *Aam11 int2 red114 cI857*; this strain carries mutations in genes *cI*, *int*, and *red* and an amber-

suppressible (*am*) mutation in gene *A*. A strain designated  $\lambda$  *att*<sup>434</sup> *imm*<sup>21</sup> would represent a hybrid of phage  $\lambda$  which carries the immunity region (*imm*) of phage 21 and the attachment (*att*) region of phage 434. Host DNA insertions into viruses should be delineated by square brackets, and the genetic symbols and designations for such inserted DNA should conform to those used for the host genome. Genetic symbols for phage  $\lambda$  can be found in reports by Szybalski and Szybalski (Gene 7:217–270, 1979) and Echols and Murialdo (Microbiol. Rev. 42:577–591, 1978).

**Eukaryotes.** For information about the genetic nomenclature of eukaryotes, see the Instructions to Authors for *Eukaryotic Cell and Molecular and Cellular Biology*.

**Transposable elements, plasmids, and restriction enzymes.** Nomenclature of transposable elements (insertion sequences, transposons, phage Mu, etc.) should follow the recommendations of Campbell et al. (Gene 5:197–206, 1979), with the modifications given in section vi above. The Internet site where insertion sequences of eubacteria and archaea are described and new sequences can be recorded is <http://www-is.biotoul.fr/is.html>.

The system of designating transposon insertions at sites where there are no known loci, e.g., *zef-123::Tn5*, has been described by Chumley et al. (Genetics 91:639–655, 1979). The nomenclature recommendations of Novick et al. (Bacteriol. Rev. 40:168–189, 1976) for plasmids and plasmid-specified activities, of Low (Bacteriol. Rev. 36:587–607, 1972) for F' factors, and of Roberts et al. (Nucleic Acids Res. 31:1805–1812, 2003) for restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes should be used whenever possible. The nomenclature for recombinant DNA molecules, constructed in vitro, follows the nomenclature for insertions in general. DNA inserted into recombinant DNA molecules should be described by using the gene symbols and conventions for the organism from which the DNA was obtained.

**Tetracycline resistance determinants.** The nomenclature for tetracycline resistance determinants is based on the proposal of Levy et al. (Antimicrob. Agents Chemother. 43:1523–1524, 1999). The style for such determinants is, e.g., Tet B; the space helps distinguish the determinant designation from that for phenotypes and proteins (TetB). The above-referenced article also gives the correct format for genes, proteins, and determinants in this family.

## ABBREVIATIONS AND CONVENTIONS

### Verb Tense

ASM strongly recommends that for clarity you use the **past** tense to narrate particular events in the past, in-

cluding the procedures, observations, and data of the study that you are reporting. Use the present tense for your own general conclusions, the conclusions of previous researchers, and generally accepted facts. Thus, most of the abstract, Materials and Methods, and Results will be in the past tense, and most of the introduction and some of the Discussion will be in the present tense.

Be aware that it may be necessary to vary the tense in a single sentence. For example, it is correct to say "White (30) demonstrated that XYZ cells grow at pH 6.8," "Figure 2 shows that ABC cells failed to grow at room temperature," and "Air was removed from the chamber and the mice died, which proves that mice require air." In reporting statistics and calculations, it is correct to say "The values for the ABC cells are statistically significant, indicating that the drug inhibited. . ."

For an in-depth discussion of tense in scientific writing, see p. 191–193 in *How To Write and Publish a Scientific Paper*, 6th ed.

## Abbreviations

**General.** Abbreviations should be used as an aid to the reader, rather than as a convenience to the author, and therefore their use should be limited. Abbreviations other than those recommended by the IUPAC-IUB (*Biochemical Nomenclature and Related Documents*, 1992) should be used only when a case can be made for necessity, such as in tables and figures.

It is often possible to use pronouns or to paraphrase a long word after its first use (e.g., "the drug" or "the substrate"). Standard chemical symbols and trivial names or their symbols (folate, Ala, Leu, etc.) may also be used.

It is strongly recommended that all abbreviations except those listed below be introduced in the first paragraph in Materials and Methods. Alternatively, define each abbreviation and introduce it in parentheses the first time it is used; e.g., "cultures were grown in Eagle minimal essential medium (MEM)." Generally, eliminate abbreviations that are not used at least three times in the text (including tables and figure legends).

**Not requiring introduction.** In addition to abbreviations for Système International d'Unités (SI) units of measurement, other common units (e.g., bp, kb, and Da), and chemical symbols for the elements, the following should be used without definition in the title, abstract, text, figure legends, and tables: DNA (deoxyribonucleic acid); cDNA (complementary DNA); RNA (ribonucleic acid); cRNA (complementary RNA); RNase (ribonuclease); DNase (deoxyribonuclease); rRNA (ribosomal RNA); mRNA (messenger RNA); tRNA (transfer RNA); AMP, ADP, ATP, dAMP, ddATP, GTP, etc. (for the respective 5' phosphates of adenosine and other nucleosides) (add 2', 3', or 5' when needed for contrast); ATPase, dGTPase, etc. (adenosine triphosphatase, deoxyguanosine triphosphatase, etc.); NAD (nicotinamide adenine dinucleotide); NAD<sup>+</sup> (nicotinamide adenine dinucleotide, oxidized); NADH (nicotinamide adenine dinucleotide, reduced); NADP (nicotinamide

adenine dinucleotide phosphate); NADPH (nicotinamide adenine dinucleotide phosphate, reduced); NADP<sup>+</sup> (nicotinamide adenine dinucleotide phosphate, oxidized); poly(A), poly(dT), etc. (polyadenylic acid, polydeoxythymidylic acid, etc.); oligo(dT), etc. (oligodeoxythymidylic acid, etc.); UV (ultraviolet); PFU (plaque-forming units); CFU (colony-forming units); MIC (minimal inhibitory concentration); Tris [tris(hydroxymethyl)aminomethane]; DEAE (diethylaminoethyl); EDTA (ethylenediaminetetraacetic acid); EGTA [ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid]; HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid); PCR (polymerase chain reaction); and AIDS (acquired immunodeficiency syndrome). Abbreviations for cell lines (e.g., HeLa) also need not be defined.

The following abbreviations should be used without definition in tables:

amt (amount)	SE (standard error)
approx (approximately)	SEM (standard error of the mean)
avg (average)	
concn (concentration)	sp act (specific activity)
diam (diameter)	sp gr (specific gravity)
expt (experiment)	temp (temperature)
exptl (experimental)	tr (trace)
ht (height)	vol (volume)
mo (month)	vs (versus)
mol wt (molecular weight)	wk (week)
no. (number)	wt (weight)
prepn (preparation)	yr (year)
SD (standard deviation)	

**Drugs and pharmaceutical agents.** Should an author decide to abbreviate the names of antimicrobial agents in a manuscript, the following standard abbreviations are strongly recommended.

(i) *Antibacterial agents.* Amikacin, AMK; amoxicillin, AMX; amoxicillin-clavulanic acid, AMC; ampicillin, AMP; ampicillin-sulbactam, SAM; azithromycin, AZM; azlocillin, AZL; aztreonam, ATM; carbenicillin, CAR; cefaclor, CEC; cefadroxil, CFR; cefamandole, FAM; cefazolin, CFZ; cefdinir, CDR; cefditoren, CDN; cefepime, FEP; cefetamet, FET; cefixime, CFM; cefmetazole, CMZ; cefonicid, CID; cefoperazone, CFP; cefotaxime, CTX; cefotetan, CTD; cefoxitin, FOX; cefpodoxime, CPD; cefprozil, CPR; ceftazidime, CAZ; ceftibuten, CTB; ceftizoxime, ZOX; ceftriaxone, CRO; cefuroxime (axetil or sodium), CXM; cephalixin, LEX; cephalothin, CEF; cephalirin, HAP; cephradine, RAD; chloramphenicol, CHL; cinoxacin, CIN; ciprofloxacin, CIP; clarithromycin, CLR; clinafloxacin, CLX; clindamycin, CLI; colistin, CST; daptomycin, DAP; dicloxacillin, DCX; dirithromycin, DTM; doxycycline, DOX; enoxacin, ENX; erythromycin, ERY; fleroxacin, FLE; fosfomicin, FOF; gatifloxacin, GAT; gentamicin, GEN; grepafloxacin, GRX; imipenem, IPM; kanamycin, KAN; levofloxacin, LVX; linezolid, LZD; lomefloxacin, LOM; loracarbef, LOR; meropenem, MEM; methicillin, MET; mezlocillin, MEZ; minocycline, MIN; moxalactam, MOX; moxifloxacin, MXF; nafcillin, NAF; nalidixic acid, NAL;

netilmicin, NET; nitrofurantoin, NIT; norfloxacin, NOR; ofloxacin, OFX; oxacillin, OXA; penicillin, PEN; piperacillin, PIP; piperacillin-tazobactam, TZP; polymyxin B, PMB; quinupristin-dalfopristin (Synercid), Q-D; rifabutin, RFB; rifampin, RIF; rifapentine, RFP; sparfloxacin, SPX; spectinomycin, SPT; streptomycin, STR; teicoplanin, TEC; telithromycin, TEL; tetracycline, TET; ticarcillin, TIC; ticarcillin-clavulanic acid, TIM; tigecycline, TGC; tobramycin, TOB; trimethoprim, TMP; trimethoprim-sulfamethoxazole, SXT; trovafloxacin, TVA; and vancomycin, VAN.

(ii)  *$\beta$ -Lactamase inhibitors*. Clavulanic acid, CLA; sulbactam, SUL; and tazobactam, TZB.

(iii) *Antifungal agents*. Amphotericin B, AMB; clotrimazole, CLT; flucytosine, 5FC; fluconazole, FLC; itraconazole, ITC; ketoconazole, KTC; nystatin, NYT; terbinafine, TRB; and voriconazole, VRC.

(iv) *Antiviral agents*. Acyclovir, ACV; cidofovir, CDV; famciclovir, FCV; foscarnet, FOS; ganciclovir, GCV; penciclovir, PCV; valaciclovir, VCV; and zidovudine, AZT.

**The use of "nonstandard" abbreviations to designate names of antibiotics and other pharmaceutical agents generally will not be accepted**, because the use of different abbreviations for a single agent has often caused confusion. If, on occasion, a nonstandardized abbreviation for a drug or pharmaceutical substance is used, it will be accepted under the following conditions: (i) it must be defined in an abbreviation paragraph in Materials and Methods or at the first use in the text, (ii) it must be unambiguous in meaning, and (iii) it must contribute to ease of assimilation by readers.

**Chemical or generic names of drugs should be used; the use of trade names is not permitted.** Designation of  $\beta$ -lactam antibiotics by generation is discouraged. **When code names or corporate proprietary numbers are to be used, either the chemical structure of the compound or a published literature reference illustrating the chemical structure, if known, must be provided at the first occurrence of the code name or number. For compounds not identified by generic nomenclature, all previous or concurrent identification numbers or appellations should be listed in the manuscript.**

**Pharmacokinetic parameters.** Abbreviations and symbols for pharmacokinetic parameters must be introduced at their first occurrence in the text. Those most commonly used are  $\alpha$  (or  $\alpha$  phase), distribution phase;  $\beta$  (or  $\beta$  phase), elimination phase;  $A$ , zero-time intercept for  $\alpha$  phase;  $B$ , zero-time intercept for  $\beta$  phase; AUC, area under the concentration-time curve; AUMC, area under the first moment of the concentration-time curve;  $AUC_{0-24}$ ,  $AUC_{0-\infty}$ , etc., area under the concentration-time curve from 0 to 24 h, 0 h to  $\infty$ , etc.; CL, clearance;  $CL_R$ , renal clearance;  $CL_{NR}$ , nonrenal clearance;  $CL_{CR}$ , creatinine clearance;  $C_{max}$ , maximum concentration of drug in serum;  $T_{max}$ , time to maximum concentration of

drug in serum;  $V_{max}$ , maximum rate of metabolism;  $X_u^{1-2}$ , drug concentration in urine between  $t_1$  and  $t_2$ ;  $V$ , volume of distribution;  $V_{ss}$ , volume of distribution at steady state;  $V_1$ , volume of distribution of the central compartment;  $k_{el}$ , elimination rate constant;  $k_{ss}$ , residence rate constant at steady state;  $t_{1/2}$ , half-life;  $t_{1/2\alpha}$ , half-life at  $\alpha$  phase; and  $t_{1/2\beta}$ , half-life at  $\beta$  phase. For other symbols, see Rowland and Tucker (J. Pharmacokin. Biopharm. 8:497-507, 1980).

**Pharmacodynamic terminology.** Pharmacodynamic indices (PDIs) must be introduced at their first occurrence in the text and follow guidelines set forth by Mouton et al. (J. Antimicrob. Chemother. 55:601-607, 2005). In Materials and Methods, it should be clearly stated how the PDIs were derived. The most common indices used are the following: AUC/MIC ratio (the area under the concentration-time curve over 24 h in steady state divided by the MIC), AUIC (the area under the inhibitory curve; note that the AUC/MIC ratio is not equal to the AUIC),  $\%T_{MIC}$  (the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC at steady-state pharmacokinetic conditions),  $C_{max}/MIC$  ratio (the peak level divided by the MIC), PTA (probability of target attainment), and CFR (cumulative fraction of response). Clear distinction should be made between  $\%T_{MIC}$ , which is expressed as a percentage of the dosing interval, and  $T_{MIC}$ , expressed in hours. It is strongly recommended that the prefix  $f$  be used with an index (e.g.,  $fAUC$ ) if the free, unbound fraction of the drug is meant.

### $\beta$ -Lactamases

Studies performed to characterize a  $\beta$ -lactamase or the interaction of a compound with a  $\beta$ -lactamase (i.e., as a substrate, inhibitor, or inducer) should follow the guidelines set forth by Bush and Sykes (Antimicrob. Agents Chemother. 30:6-10, 1986). Assays that measure the hydrolysis of  $\beta$ -lactam antibiotics must be appropriate for the substrate examined (e.g., iodometric methods are not appropriate quantitative assays for substrates whose products are unknown). Reproducibility of results must be shown. When referring to  $\beta$ -lactamases, please use the functional designations defined by Bush et al. (Antimicrob. Agents Chemother. 39:1211-1233, 1995). Alternatively, if the amino acid sequence for the enzyme is known, the  $\beta$ -lactamases may be described by molecular class as initiated by Ambler (Phil. Trans. R. Soc. Lond. 289:321-331, 1980).

A database of defining amino acid alterations for new  $\beta$ -lactamases is maintained at the Internet address <http://www.lahey.org/studies/> and is also accessible via the ASM home page at <http://www.asm.org>. It should be consulted before a new designation is proposed.

### In Vitro Susceptibility Tests

Tabulate results of determinations of minimal inhibitory and bactericidal concentrations according to the

range of concentrations of each antimicrobial agent required to inhibit or kill the members of a species or of each group of microorganisms tested, as well as the corresponding concentrations required to inhibit 50 and 90% of the strains ( $MIC_{50}$  and  $MIC_{90}$ , respectively) and those required to kill 50 and 90% of the strains ( $MBC_{50}$  and  $MBC_{90}$ , respectively). The  $MIC_{50}$  and  $MIC_{90}$  reported should be the actual concentrations tested that inhibited 50 and 90%, respectively, of the strains. They should not be values calculated from the actual data obtained. When only six to nine isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested.

If more than a single drug is studied, insert a column labeled "Test agent" between the columns listing the organisms and the columns containing the numerical data and record data for each agent in the same isolate order. Cumulative displays of MICs or MBCs in tables or figures are acceptable only under unusual circumstances.

The percentage of strains susceptible and/or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by the Clinical and Laboratory Standards Institute, 940 W. Valley Rd., Suite 1400, Wayne, PA 19087-1898. In the absence of approved breakpoints, authors cannot assign breakpoints or use breakpoints from related antibiotics. An exploratory analysis tabulating the percentage of strains inhibited over a range of concentrations is acceptable.

Bactericidal tests must be performed with a sufficient inoculum ( $>5 \times 10^5$  CFU/ml) and subculture volume (0.01 ml) to ensure accurate determination of the 99.9% killing endpoint, as described by Pearson et al. (Antimicrob. Agents Chemother. 18:699-708, 1980) and Taylor et al. (Antimicrob. Agents Chemother. 23:142-150, 1983). Inoculum size and subculture volume are also critical to studies of combinations of antimicrobial agents.

Synergy is defined in two-dimensional or checkerboard tests when the fractional inhibitory concentration (FIC) or fractional bactericidal concentration (FBC) index ( $\Sigma$ ) is  $\leq 0.5$ . In killing curves, synergy is defined as a  $\geq 2$ -log<sub>10</sub> decrease in CFU per milliliter between the combination and its most active constituent after 24 h, and the number of surviving organisms in the presence of the combination must be  $\geq 2$  log<sub>10</sub> CFU/ml below the starting inoculum. At least one of the drugs must be present in a concentration which does not affect the growth curve of the test organism when used alone. Antagonism is defined by a  $\Sigma FIC$  or  $\Sigma FBC$  of  $>4.0$ .

When standard twofold dilution schemes are used to determine checkerboard interactions, the inherent variability of the method casts doubt on the significance of interactions represented by  $\Sigma FICs$  or  $\Sigma FBCs$  of  $>0.5$  but  $\leq 4$ . Therefore, such interactions, if labeled at all, should be termed "indifferent." Alternatively, indices in this range may be described as "nonsynergistic" or "nonantagonistic," as appropriate. The technically imprecise term "additive" should be avoided as it is too easily misunderstood. See reports by W. R. Greco et al. (Phar-

macol. Rev. 47:331-385, 1995), F. C. Odds (J. Antimicrob. Chemother. 52:1, 2003), and M. D. Johnson et al. (Antimicrob. Agents Chemother. 48:693-715, 2004) for further discussion of these issues.

For killing curve tests, the minimal, accurately countable number of CFU per milliliter must be stated and the method used for determining this number must be described. In the absence of any drug and with a sample size of 1 ml, this number is 30 (1.5 in log<sub>10</sub>) CFU. If procedures for drug inactivation or removal have not been performed, the author must state how drug carryover effects were eliminated or quantified. For drugs showing an inoculum effect, mere dilution below the MIC obtained in standard tests is not sufficient.

### Clinical Trials

(i) **Criteria for enrollment.** The methods used to find and enroll patients and the criteria for enrollment in a clinical trial should be stated. In addition, the time period (month/year to month/year) of the enrollment should be specified. It should be indicated, if appropriate, that written informed consent was obtained and that the trial was approved by the pertinent committee on human subjects.

(ii) **Method of randomization.** Randomized, double-blind studies are preferred. Comparisons using historical controls are usually regarded as questionable unless the differences in outcome between the groups are dramatic and almost certainly the result of the new intervention. The rationale for the choice of the control group should be explained. The sample size should be justified, and the method of randomization should be stated.

(iii) **Criteria for determining whether a case is evaluable.** The minimum criteria for evaluability should be stated explicitly. For example, it should be stated that the minimum criterion for evaluability was *a* or the combination of *b* and *c* rather than *a*, *b*, and *c* without designating which were the minimum criteria. The criteria for evaluability are usually different from those for enrollment.

(iv) **Reasons for nonevaluability.** State the number of patients in each group who were excluded from evaluation and the reason(s) for each exclusion.

(v) **Criteria for assessment.** Define each outcome for each category of assessment (e.g., "clinical outcomes were classified as cure, improvement, and failure; microbiological outcomes were classified as eradication, persistence, and relapse"). The frequency and timing of such assessments in relation to treatment should be stated. Specify any changes made in the study regimen(s) during the trial; the results for regimens with and without such modification generally should be stated separately. The criteria (questionnaires, results of specific laboratory tests) for evaluation of adverse ef-

fects should be stated, as should the period encompassed in the assessment and the time of assessment in relation to the time of treatment (e.g., daily during treatment). Some authors prefer to consider superinfections as failures of treatment, whereas others prefer to consider them separately or even as adverse effects. In any event, the manuscript should state the number of superinfections with each regimen and should differentiate between superinfections and colonization. The duration of follow-up should be mentioned.

**(vi) Statistical analyses.** The type of statistical test should be stated and, when appropriate, the reason for the choice of test should be given. References should be given for statistical procedures other than the *t* test, chi-square test, and Wilcoxon rank sum test. The comparability of the treatment groups at the baseline should be evaluated statistically.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the article by C. Olsen (*Infect. Immun.* 71:6689–6692, 2003).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (*J. Virol.* 79:669–676, 2005).

**(vii) Beta error.** For trials which show no statistically significant difference between regimens, the authors should calculate the probability ( $\beta$ ) of a type II error and the power of the study ( $1 - \beta$ ) to detect a specified clinically meaningful difference in efficacy between the regimens. For further details, see Freiman et al. (*N. Engl. J. Med.* 299:690–694, 1978). Alternatively, or in addition, the authors should indicate the magnitude of difference between the regimens that could have been detected at a statistically significant level with the number of evaluable patients studied.

For further details, see the editorial on guidelines for clinical trials (*Antimicrob. Agents Chemother.* 33:1829–1830, 1989).

## Reporting Numerical Data

Standard metric units are used for reporting length, weight, and volume. For these units and for molarity, use the prefixes m,  $\mu$ , n, and p for  $10^{-3}$ ,  $10^{-6}$ ,  $10^{-9}$ , and  $10^{-12}$ , respectively. Likewise, use the prefix k for  $10^3$ . Avoid compound prefixes such as m $\mu$  or  $\mu\mu$ . Use  $\mu\text{g/ml}$

or  $\mu\text{g/g}$  in place of the ambiguous ppm. Units of temperature are presented as follows: 37°C or 324 K.

When fractions are used to express units such as enzymatic activities, it is preferable to use whole units, such as g or min, in the denominator instead of fractional or multiple units, such as  $\mu\text{g}$  or 10 min. For example, “pmol/min” is preferable to “nmol/10 min,” and “ $\mu\text{mol/g}$ ” is preferable to “nmol/ $\mu\text{g}$ .” It is also preferable that an unambiguous form such as exponential notation be used; for example, “ $\mu\text{mol g}^{-1} \text{min}^{-1}$ ” is preferable to “ $\mu\text{mol/g/min}$ .” Always report numerical data in the appropriate SI units.

Representation of data as accurate to more than two significant figures must be justified by presentation of appropriate statistical analyses.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the article by Olsen (*Infect. Immun.* 71:6689–6692, 2003).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (*J. Virol.* 79:669–676, 2005).

## Isotopically Labeled Compounds

For simple molecules, labeling is indicated in the chemical formula (e.g.,  $^{14}\text{CO}_2$ ,  $^3\text{H}_2\text{O}$ , and  $\text{H}_2^{35}\text{SO}_4$ ). Brackets are not used when the isotopic symbol is attached to the name of a compound that in its natural state does not contain the element (e.g.,  $^{32}\text{S-ATP}$ ) or to a word that is not a specific chemical name (e.g.,  $^{131}\text{I}$ -labeled protein,  $^{14}\text{C}$ -amino acids, and  $^3\text{H}$ -ligands).

For specific chemicals, the symbol for the isotope introduced is placed in square brackets directly preceding the part of the name that describes the labeled entity. Note that configuration symbols and modifiers precede the isotopic symbol. The following examples illustrate correct usage:

$^{14}\text{C}$ urea	UDP- $^{14}\text{C}$ glucose
L-[methyl- $^{14}\text{C}$ ]methionine	<i>E. coli</i> [ $^{32}\text{P}$ ]DNA
[2,3- $^3\text{H}$ ]serine	fructose 1,6-[1- $^{32}\text{P}$ ]bisphosphate
$[\alpha\text{-}^{14}\text{C}]$ lysine	
$[\gamma\text{-}^{32}\text{P}]$ ATP	

AAC follows the same conventions for isotopic labeling as the *Journal of Biological Chemistry*, and more-detailed information can be found in the instructions to authors of that journal (first issue of each year).